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LONGEVITY OF THE TELIOSPORES AND ACCOMPANYING UREDOSPORES OF CRONARTIUM RIBICOLA FISCHER IN 1923 [1]

By PERLEY SPAULDING, *Pathologist*, and ANNIE RATHBUN-GRAVATT, *Junior Pathologist, Office of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

This paper gives the results of investigations of an isolated problem connected with the life history of the white-pine blister rust. It may not be amiss, therefore, to indicate its relation to the general problem. These investigations show that the teliospores, which give rise to the sporidia (which alone can infect pines), may survive outdoor conditions from the time of the formation of the first ones in the season until after winter has set in. Little is yet known of the period during which pines may be infected, but it is presumed that it includes the period of telial formation and possible germination. It is important, then, to know how long and how late in the season teliospores may survive and form sporidia, and the probability of their doing so.

In the late fall and early winter of 1921 the senior writer tested the longevity of the teliospores of *Cronartium ribicola* Fischer (*16*).[2] While the results were satisfactory for the period covered (September 21 to December 8), it was felt that such tests should begin earlier in the season of production of the teliospores (*17, p. 63*) in order to get an idea of the maximum telial longevity under natural conditions. What may happen to the teliospores which are formed earliest in the season has received little direct attention, and this may be of considerable importance. Accordingly, in 1923 the writers made another study with this especially in mind. These tests began August 1, which was the earliest date at which a large enough supply of telial columns could be obtained, and continued until October 31. From August 1 to October 10 the work was carried on at Warrensburg, N. Y., and from October 10 to 31 at Bethel, Vt., under similar conditions. When the experiments were discontinued on October 31 the teliospores in several lots of material were still germinating strongly. The work of 1921 (*16*) showed conclusively that teliospores of this fungus will readily survive and germinate under the conditions of early winter in Vermont. Judging from the vigor of their germination and the fact mentioned in the preceding sentence, there was no reason to suppose that these persisting teliospores in the present experiments would lose their viability for several weeks after October 31. In view of these conclusions and the pressure of other work which required that these tests be concluded as soon as possible, no attempt was made to continue until the viability of all of the teliospores was exhausted.

[1] Received for publication Mar. 3, 1925; issued January, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 1015.

THE MATERIAL

CHARACTER OF THE MATERIAL

Twenty-one collections of leaves bearing abundant telial columns were obtained from eight species of Ribes (see Table IV for their names)[3] in various parts of New Hampshire and northern New York. This material was about as unfavorable for our purpose as could ordinarily be obtained, because in some places there were prolonged local droughts which resulted in sparse telial production and prevented obtaining freshly matured columns (*17, p. 63*). Pennington (*13*) in 1920, because of the evenly scattered distribution of the rains during that summer, could get but few viable sporidia of *Cronartium ribicola*, in an area which generally is heavily infected, before the first week of September. In other places it rained frequently enough to partially germinate the telial columns as soon as they were formed.

The amount of pregermination (i. e., germination previous to the collection of the infected leaves) of the telial columns varied greatly. The average pregermination of the different collections of leaves ranged from 0 to 60 per cent. In most cases it was below 15 per cent. On the different leaves of a given collection the pregermination often varied from 0 to 100 per cent of all of the telial columns examined. There was also a similar variation in the average pregermination of the telial columns on the different parts of a single leaf. A telial column which showed one or more germinated teliospores was counted as pregerminated. In such a column there might be left a thousand (*19*) or more teliospores which were perfectly viable when placed under favorable conditions for germination in the subsequent tests.

In damp-chamber tests made immediately after collection of the material, the average germinability of the telial columns (based upon the column as a unit) varied in the different collections from 7 to 97 per cent. In more than half of the collections it was over 70 per cent. The viability of the teliospores on different leaves and on different parts of the same leaf varied just as did the pregermination for the same units.

While these conditions hindered the writers in getting entirely consistent results, and certainly prevented the obtaining of maximum longevity, they also forestall any criticism for having worked with unduly favorable material.

CONDITIONS DURING STORAGE OF THE MATERIAL

The infected Ribes leaves were taken to the field laboratory as soon as feasible after collection. There they were laid singly upon newspapers and allowed to dry in the open air, where they were protected from direct sunshine. As soon as the leaves were wilted, but not crisp, they were placed in mosquito-netting bags and hung out of doors fully exposed to the rain and wind but not to very much sunshine. After rains, when the leaves had begun to dry, they were shaken up so that they would not become matted into a solid mass. An attempt was made to simulate the conditions affecting naturally fallen leaves which had caught among shrubbery in the forest.

[3] The nomenclature followed is that given in Coville and Britton (*6*).

At Warrensburg, N. Y., rain fell in quantities sufficient to wet the foliage on 26 different days, while at Bethel, Vt., this occurred on but 2 days. At the former place there were 7 periods of more than 36 hours when the relative humidity of the air remained above 70 per cent. This condition is shown by the work of the writers to be favorable for the sporidia to retain viability for a long time, and is discussed in their paper on the sporidia of *Cronartium ribicola*.[4]

SAMPLING THE MATERIAL

In order to have representative material for each test, about half of the leaves used in each sample were taken from the middle of the storage bag and the remainder from the outside. The samples were taken after the dew had evaporated. Each sample was placed in a clean, dry Petri dish.

PRECOOLING THE MATERIAL BEFORE TESTING IT

Because it has been generally supposed that precooling per se stimulates the germination of the spores of the Uredineae (*7*, *8*, *9*, *10*, *16*, *17*, *21*), it was thought best to precool the material before starting germination of the teliospores. In the first two tests this was done by placing the Petri dishes containing the leaf samples in an ordinary refrigerator for about 24 hours. The material for the other tests, with the exceptions noted in Table IV, was placed in the Petri dishes and these then put in a metal container, which was packed in cracked ice and coarse salt for about 24 hours. The temperature thus obtained was certainly lower than that in the refrigerator, although no records were kept. The nightly hard frosts, which began about October 10, were considered to have the desired cooling effect, and the material was not further precooled in the tests run after that date. There was a marked increase in germination of some of the material beginning October 17, the date of the first test run after the frosts began. A suggested explanation is given below.

Table I gives a comparison of germination of precooled and of nonprecooled telial columns. The results are fragmentary because the work was done late in the season, when suitable material was scarce; earlier it was supposed that the effect of precooling was thoroughly established by other workers, and the need for further testing this point was not realized. In the tests recorded in Table I, half of the tested material was precooled by packing the Petri dishes containing it in a mixture of salt and ice for about 24 hours; the other half of the material was kept at room temperature for the same length of time. The two methods of comparing the germination of the two samples of material show that the germination was practically equal in the first two lots of Table I which are designated there as "Fresh telial columns" and "Old telial columns stored indoors." In the lot designated as "Old telial columns stored outdoors," the same methods of comparison show that the uncooled telial columns germinated better; that is, those stored at relatively low temperatures and then subjected to higher temperatures reacted

[4] SPAULDING, P., and GRAVATT, A. KATHRUN. THE INFLUENCE OF PHYSICAL FACTORS UPON THE VIABILITY OF THE SPORIDIA OF CRONARTIUM RIBICOLA FISCHER, 1925. [Unpublished manuscript.]

more decidedly than those from a part of the same material in which the low temperatures were followed by still lower ones. So far as these tests go, the writers find that there is no indication that precooling per se of the teliospores stimulates their subsequent germinability. On the contrary, there is some indication that the effect is rather that of decided variation of the temperatures. This suggestion is further supported by the increases in germination which resulted in the tests beginning October 17, apparently as a result of repeated sharp alternation of high daytime and low night temperatures during a period of three weeks with but two days of rain. Eriksson, who seems to have been the first to test the effect of precooling on the spores of *Cronartium ribicola* (*9*), got a decided reaction as a result of his treatment of the spores, but he does not definitely attribute it to precooling per se or to variation of the temperature. He inclines to the idea that rain just before the spores are collected for cooling has a deciding influence in the reaction obtained.

TABLE I.—*Comparison of the germination of precooled and uncooled telial columns of Cronartium ribicola*

Condition of material at the time of testing	Total number of tests	Number of tests in which precooled telial columns germinated better than uncooled ones	Number of tests in which precooled telial columns germinated poorer than uncooled ones	Number of tests in which neither germinated	Actual germination			
					Precooled telial columns		Uncooled telial columns	
					Number	Per cent	Number	Per cent
Fresh telial columns	9	5	4	0	* 525/1065	49	406/930	44
Old telial columns stored indoors	11	4	5	2	95/1752	5	106/1783	6
Old telial columns stored outdoors	13	0	11	2	210/2148	10	540/2163	25

* Numerators indicate number of columns which germinated; denominators, the number counted.

The writers' results are merely suggestive. The effects of precooling per se, of decided temperature changes, and of sharp alternations of temperatures on fresh material, on material stored at cold temperatures, and on material stored at warm temperatures should be investigated further.

GERMINATION OF THE TELIOSPORES

OBSERVATIONS ON THE GERMINATION OF THE TELIOSPORES[1]

The telial columns and the teliospores are distinct units, and there is no relation between the percentage of telial columns which show germination and the number of teliospores per column which germinate. Throughout the tables in this paper the numerators of the fractions indicate the number of columns which germinated, while the denominators show the total number of columns counted. In

[1] The citations in this chapter are arranged chronologically.

columns 4, 7, etc., of Table IV, and in similar columns of our other tables, X indicates that less than one-third of the visible teliospores in the column germinated; XX indicates that between one-third and two-thirds of the visible teliospores germinated; while XXX indicates that more than two-thirds of them germinated. This method of estimating the number of germinating teliospores is not satisfactory, but no really desirable method of doing this has yet been devised (*10, 17*).

While the present paper is concerned primarily with the longevity of the teliospores, the writers' observations on their manner of germination, etc., may well be mentioned here. The following phenomena have been noted more or less frequently in these tests. They are not new to the writers, and the references indicate others who have made similar observations. They are chosen especially for work with *Cronartium ribicola*, and in default of this, with some other species of Cronartium.

The teliospores germinate normally in situ in the telial column, and with the latter still upon the host leaf (*15, 5, 4*). The basal spores in a telial column are the youngest, and germination proceeds in each column from the tip downward as the teliospores mature (*20, 15, 5*). For this reason we may have a telial column at the tip of which are teliospores which have germinated, and still have in it other teliospores which will readily germinate when the necessary favorable conditions are furnished. Each teliospore normally puts forth a germ tube. If the tube immediately reaches the air, it soon divides into a four-celled promycelium (*20, 14, 15, 5, 4, 17*). If the teliospore is immersed in water, it may fail to germinate at all, or may produce a long, slender tube, which appears to try to reach the air (*5, 4*). Sometimes this tube forms a four-celled structure at its end, the cells of which soon fall apart, forming what are evidently resting spores (*15, 4*). If the germ tube from the teliospore should reach the air, it immediately forms a four-celled promycelium. Air seems to be necessary for the formation of normal promycelia. As the germ tube elongates from the teliospore, and especially when the promycelium is formed, the protoplasm of the teliospore enters the tube and fills its outer end (*20, 15, 5, 4*). The four cells of the promycelium receive this protoplasmic mass, and when they in turn germinate the protoplasm migrates to the ends of their germ tubes. If secondary or tertiary sporidia are formed, the protoplasm continues to migrate to the outermost structure, leaving those behind empty. Normally each promycelial cell germinates, forming a germ tube. This soon forms a small, round conidium called a sporidium (*20, 14, 15, 5, 4*). The sporidium germinates by putting forth a germ tube; this may quite frequently produce a secondary sporidium nearly as large as the primary one. Sometimes the process is repeated and a tertiary sporidium formed (*14, 15, 21, 5, 4, 17*). In the experiments described in this paper, secondary sporidia were noted in cultures of teliospores from *Ribes americanum*, *R. glandulosum*, *R. nigrum*, *R. rotundifolium*, *R. triste*, and *R. vulgare*. They were not noted in material from *R. cynosbati* and *R. odoratum*.

In some of their tests the writers noted the effect of massing of the telial columns in decreasing the germination of the teliospores, which is mentioned by Brown (*3*) for spores of *Botrytis cinerea*.

That is, it was noticed that germination occurred only around the edges of masses of columns. This did not hold in all cases, but was frequent enough to attract attention.

METHODS OF GERMINATION

The original plan was to germinate the teliospores by placing the Ribes leaves, bearing the telial columns, upside down in moist chambers until the teliospores had germinated, and then to scrape off the columns for examination, as had been done previously (*16*). However, a series of 29 parallel tests showed that the "floating" method of germination described below gave decidedly better results than did the moist-chamber method. (See Table II.) In but one instance was germination in the moist chamber better than germination by the floating method. Therefore the floating method was adopted and used for practically the entire series of experiments.

TABLE II.—*Comparison of germination of telial columns of Cronartium ribicola by the "moist-chamber" and "floating-on-water" methods*

Ribes species	Number of collections	Number of tests	Number of days on which tests were made	Number of times better	Germination	
					Number	Per cent
Americanum:						
Water	2	4	4	4	204/222	92
Moist air	2	4	4	0	235/530	44
Cynosbati:						
Water	1	2	2	2	93/142	66
Moist air	1	2	2	0	48/455	11
Glandulosum:						
Water	2	3	2	3	343/375	92
Moist air	2	3	2	0	69/410	17
Nigrum:						
Water	4	14	5	13	886/1,458	61
Moist air	4	14	5	1	457/3,000	15
Odoratum:						
Water	1	2	2	2	145/185	78
Moist air	1	2	2	0	29/480	6
Rotundifolium:						
Water	1	2	1	2	152/205	74
Moist air	1	2	1	0	57/225	25
Vulgare:						
Water	1	2	1	2	172/180	96
Moist air	1	2	1	0	12/120	10
Total:						
Water	12	29	7	28	1,995/2,767	72
Moist air	12	29	7	1	907/5,220	17

The floating method of germinating spores was not originated by the writers (*14, 1, 2, 9, 3, 11*).[6] It is apparently an outgrowth of the hanging-drop method. Most writers who have used the hanging-drop method do not clearly state whether the spores floated on the surface of the liquid or were immersed in it in their experiments, but a few clearly differentiated between the two (*14, 1, 15, 7*). Barclay (*1, 2*) seems to be the first to definitely state that he floated the spores on the surface of the water. H. H. York was the first to use it for spores of *Cronartium ribicola*.

In these experiments a surgeon's cataract knife, with an arrow-head shaped, double-edged blade not more than one-fourth inch long, was

[6] In this paragraph the citations are arranged chronologically.

found best for scraping the columns from the leaves to the surface of water in small glasses containing 10 to 35 c. c. each, according to their size. The telial columns for each glass of water were taken from all of the leaves of a given sample. The leaves should be slightly damp so as to be pliable without breaking, but not wet enough so that the knife edge will scrape water from the leaf tissue, as this will wet the columns and they will sink in the water when dropped on the surface of it. When they sink they germinate little or not at all, as is true of rust spores in general (*1*, *2*, *3*, *7*, *10*, *11*, *14*). But if dry, they will float readily and for a long time.

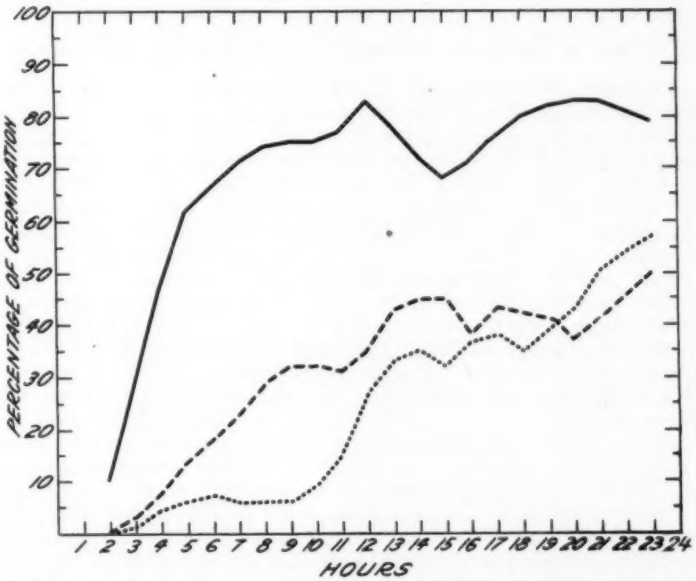

FIG. 1.—Diagram showing time necessary in preliminary tests for the germination of telial columns of *Cronartium ribicola*. The curves are based on 3-hour moving averages, and represent the per cent of the total number of telial columns germinating. The solid line represents the results from telial columns produced on *Ribes nigrum* stored indoors 5 days. The dash line represents those from *R. americanum* stored indoors 15 days. The dotted line represents those from *R. nigrum* stored indoors 25 days

LENGTH OF THE GERMINATION PERIOD

The teliospores were germinated outdoors, shielded from sun and rain, for a period of 16 to 18 hours. Under the conditions of this work, this length of time was necessary for the maximum amount of germination to take place. This period was unexpectedly long in comparison with 6 hours required in previous years to germinate freshly matured teliospores (*22*, *17*).

Some tests were made to determine the hourly germination of telial columns which had been stored for various lengths of time. Table III and Figure 1 give the results of these tests. The writers' experience shows plainly that the time necessary for germination increases directly with length of time of storage in a dry condition.

TABLE III.—*Time necessary for germination of teliospores of Cronartium ribicola*

Ribes species	Collected		Age (days) when tested	Hours to first germination	Hours to maximum germination of columns
	Place	Date, 1923			
Nigrum	Edinburg, N. Y.	Sept. 30	5	2	12
Americanum	Warrensburg, N. Y.	Sept. 18	15	4	14
Odoratum	Darrowsville, N. Y.	Sept. 12	20	4	18
Nigrum	Edinburg, N. Y.	Sept. 7	25	4	23
Do	Wadhams, N. Y.	Aug. 23	29	5	23

EFFECT OF ALTERNATE WETTING AND DRYING ON THE TELIOSPORES

A survey of the literature shows that considerable work has been done on the effect of alternate wetting and drying upon the teliospores of various genera of the rusts (*11*). But it is also found that practically all of this work has been done with those teliospores which require a resting period before germinating. Experiments with teliospores which germinate upon maturing, and especially with teliospores of any species of Cronartium, seem to be scarce. Since the writers' material was exposed to wetting by dew and especially by rain, and to the subsequent drying due to the weather conditions of the locality, this question became of some importance. The observations made by the writers seem to indicate that teliospores of *Cronartium ribicola* which do not start to germinate perceptibly are not noticeably injured by subsequent drying. When a teliospore once germinates perceptibly, drying seems to kill it. Unless teliospores start to germinate, they survive wetting and drying for an indefinite number of times, as the writers' material was wet many times, as shown above under "conditions during storage of the material." One collection of telial columns of *Ribes nigrum*, stored outdoors, germinated for 70 days while some of the same material kept indoors lived only 49 days, showing that the repeated wetting to which the former was subjected certainly did not shorten the life of the teliospores.

LONGEVITY OF THE TELIOSPORES

Teliospores from eight species of Ribes were included in these tests as compared with those from five species of Ribes in 1921 (*16*). Tables IV and V give the results of the weekly germination tests which were made to determine the longevity of the teliospores. The latter also includes the maximum longevity which was attained for each species of Ribes in 1921. The amount of germination of telial columns did not decrease regularly with increase in age in several collections of leaves. This was due partly to the variation in the amount of pregermination which had occurred and partly to irregularity in the germination which occurred while the material was kept outdoors exposed to the weather. The character of the Ribes leaves bearing the telial columns had something to do with the longevity of the teliospores (*18*).

TABLE IV.—*Longevity of teliospores of Cronartium ribicola*

[Figure following species indicates collection number]

Date tested	Ribes species, place of collection, and date collected								
	R. nigrum (1), Wevertown, N. Y., Aug. 1, 1923 (from bushes; stored inside)			R. nigrum (2), Wevertown, N. Y., Aug. 1, 1923 (from ground)			R. triste (3), Wilmington Notch, N. Y., Aug. 3, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
	Number	Per cent		Number	Per cent		Number	Per cent	
Aug. 8, 1923	[a]51	47	XXX [b]				[a]28	16	X-XXX,
	108						175		mostly XX.
Aug. 13, 1923	33	33	XX-XXX,	53	53	XXX	[a]8	9	X.
	100		mostly XX.	100			90		
Aug. 22, 1923				41	20	X-XX,	24	32	XXX.
				204		mostly X.	74		
Aug. 29, 1923				3	2—	XX	1	20	XX.
				155			5		
Sept. 5, 1923	36	10	X	9	3—	X	16	14	X-XX,
	350			370			115		mostly XX.
Sept. 12, 1923	25	17	X	13	5—	X-XX,	2	2	X.
	144			267		mostly X.	102		
Sept. 19, 1923	18	7	X-XX,	0	0	0			
	248		mostly X.	119					
Sept. 26, 1923	2	1—	X	1	1—	XX			
	255			125					
Oct. 3, 1923	[d]2	1—	X	0	0	0			
	239			130					
Oct. 10, 1923	0	0	0	0	0	0			
	165			135					

Date tested	Ribes species, place of collection, and date collected								
	R. glandulosum (4), Wilmington Notch, N. Y., Aug. 3, 1923			R. rotundifolium (5), Warrensburg, N. Y., Aug. 4, 1923			R. cynosbati (6), Warrensburg, N. Y., Aug. 6, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
	Number	Per cent		Number	Per cent		Number	Per cent	
Aug. 8, 1923	[a]13	3	X	[a]1	1—	X	33	78	XXX.
	400			255			42		
Aug. 15, 1923	29	72	X-XXX,	[a]10	4	X	60	60	XX.
	40		mostly XX.	235			100		
Aug. 22, 1923	16	38	XX	5	3	XX	9	6	XX.
	42			150			150		
Aug. 29, 1923	11	6	X-XX,	0	0	0	18	9	XX.
	175		mostly X.	95			195		
Sept. 5, 1923	39	24	X-XX,	0	0	0	15	6	X-XX,
	160		mostly X.	180			240		mostly X.
Sept. 12, 1923	5	2—	X	0	0	0	6	3+	X.
	300			180			175		
Sept. 19, 1923	11	4+	X-XXX,	0	0	0	1	1—	XXX.
	240		mostly XX.	129			202		
Sept. 26, 1923	3	2—	X				7	5—	X-XX,
	165						155		mostly XX.
Oct. 3, 1923	0	0	0				1	1+	X.
	113						94		
Oct. 10, 1923	1	1—	X				3	1	X.
	170						300		
Oct. 17, 1923 [c]	0	0	0				1	1	XX.
	20						30		
Oct. 24, 1923 [c]	0	0	0						
	12								

[a] Throughout table numerator = number of telial columns which germinated; denominator = number of telial columns which were counted.

[b] Throughout table XXX=germination of more than two-thirds of the visible teliospores; XX= germination of between one-third and two-thirds of the visible teliospores; X=germination of less than one-third of the visible teliospores.

[c] Germination in moist chambers. [d] No precooling. [e] Tests at Bethel, Vt.

TABLE IV.—*Longevity of teliospores of Cronartium ribicola*—Continued

Date tested	Ribes species, place of collection, and date collected: R. nigrum (7), Wevertown, N. Y., Aug. 6, 1923 — Germination			R. nigrum (8), Wadhams, N. Y., Aug. 6, 1923 — Germination			R. odoratum (9), Warrensburg, N. Y., Aug. 7, 1923 — Germination		
	Columns: Number	Columns: Per cent	Teliospores	Columns: Number	Columns: Per cent	Teliospores	Columns: Number	Columns: Per cent	Teliospores
Aug. 8, 1923	97	97	XXX						
	100								
Aug. 15, 1923	66	66	XXX	91	91	XXX	68	91	XXX.
	100			100			75		
Aug. 22, 1923	115	32	Mostly XXX.	94	47	XX	47	20	XX.
	360			200			235		
Aug. 29, 1923	166	54	XX-XXX, mostly XXX.	132	53	X-XXX, mostly XX.	25	17	XX.
	310			250			145		
Sept. 5, 1923	115	59	XX-XXX, mostly XXX.	19	9	X	1	1—	XXX.
	195			210			170		
Sept. 12, 1923	94	25	X-XX, mostly XX.	27	7—	XX	2	1—	X.
	375			411			292		
Sept. 19, 1923	70	24	X-XX, mostly XX.	23	9—	X-XX, mostly X.	3	1—	X.
	291			260			311		
Sept. 26, 1923	59	39	X-XXX, mostly XX.	27	14	X-XX, mostly XX.	0	0	0.
	150			191			200		
Oct. 3, 1923	41	20	XX	6	3—	X-XX, mostly X.	0	0	0.
	205			221			182		
Oct. 10, 1923	4	2+	X	2	1—	X	0	0	0.
	165			250			115		
Oct. 17, 1923 [d e]	93	33	XX	0	0	0	0	0	0.
	280			388			77		
Oct. 24, 1923 [d e]	39	14	X	0	0	0	0	0	0.
	281			280			200		
Oct. 31, 1923 [d e]	60	21	XX						
	280								

Date tested	Ribes species, place of collection, and date collected: R. nigrum (10), North Conway, N. H., Aug. 11, 1923 — Germination			R. glandulosum (11), Crawford Notch, N. H., Aug. 11, 1923 — Germination			R. triste (12), Crawford Notch, N. H., Aug. 11, 1923 — Germination		
	Columns: Number	Columns: Per cent	Teliospores	Columns: Number	Columns: Per cent	Teliospores	Columns: Number	Columns: Per cent	Teliospores
Aug. 15, 1923	[c] 0	0	0	[c] 12	14	X-XX, mostly X.	[c d] 70	70	XXX.
	100			85			100		
Aug. 22, 1923	2	20	X	5	14	XX	75	44	XXX.
	10			37			170		
Aug. 29, 1923	123	88	XXX	68	52	X-XXX, mostly XXX.	83	64	X-XXX, mostly XXX.
	140			130			130		
Sept. 5, 1923	38	18	X-XXX, mostly XX.	42	27	X-XXX, mostly XX.	41	16	X-XXX, mostly XX
	212			155			155		
Sept. 12, 1923	21	15	XX	19	12	X-XXX, mostly X.	71	24	X-XX, mostly XX.
	136			158			291		
Sept. 19, 1923	30	34	X-XXX, mostly XX.	27	15	X-XX, mostly X.	37	19	XX.
	88			177			195		
Sept. 26, 1923	3	3	X	7	6—	X	18	12	X-XXX, mostly XX.
	93			120			145		
Oct. 3, 1923							1	1—	X.
							136		
Oct. 10, 1923							0	0	0.
							225		
Oct. 17, 1923 [d e]							0	0	0.
							55		
Oct. 24, 1923 [d e]							0	0	0.
							168		

[c] Germination in moist chambers. [d] No precooling. [e] Tests at Bethel, Vt.

TABLE IV.—*Longevity of teliospores of Cronartium ribicola*—Continued

Date tested	Ribes species, place of collection, and date collected: R. americanum (13), Warrensburg, N. Y., Aug. 13, 1923 — Germination: Columns: Number	Columns: Per cent	Teliospores	R. glandulosum (14), North Hudson, N. Y., Aug. 15, 1923 — Germination: Columns: Number	Columns: Per cent	Teliospores	R. rotundifolium (15), North Hudson, N. Y., Aug. 15, 1923 — Germination: Columns: Number	Columns: Per cent	Teliospores
Aug. 15, 1923	61	91	XX-XXX,						
	67		mostly XXX.						
Aug. 22, 1923	82	58	XXX	99	43	XXX	42	41	XX.
	141			230			102		
Aug. 29, 1923	44	67	XX-XXX,	119	60	XX	70	42	X-XXX,
	66		mostly XX.	200			165		mostly XX.
Sept. 5, 1923	95	76	XX-XXX,	144	61	X-XXX,	58	24	XX.
	125		mostly XXX.	235		mostly XXX.	240		
Sept. 12, 1923	68	52	X-XXX,	21	12	X-XXX,	35	14	X-XX,
	132		mostly XX.	174		mostly X.	251		mostly XX.
Sept. 19, 1923				125	64	X-XXX,	22	10−	X-XX,
				195		mostly XX.	228		mostly X.
Sept. 26, 1923				22	11+	X-XX,	3	2+	X.
				195		mostly X.	125		
Oct. 3, 1923				32	15	X-XX,	1	1−	XX.
				215		mostly X.	187		
Oct. 10, 1923				1	1−	X	[d] 1	1	X.
				175			100		
Oct. 17, 1923 [d e]				92	43−	X	0	0	0.
				214			78		
Oct. 24, 1923 [d e]				8	3	XX	0	0	0.
				259			248		
Oct. 31, 1923 [d e]				18	5−	X			
				378					

Date tested	Ribes species, place of collection, and date collected: R. vulgare (16), North Hudson, N. Y., Aug. 16, 1923 — Germination: Columns: Number	Columns: Per cent	Teliospores	R. americanum (17), Warrensburg, N. Y., Aug. 20, 1923 — Germination: Columns: Number	Columns: Per cent	Teliospores	R. odoratum (18), Westport, N. Y., Aug. 23, 1923 — Germination: Columns: Number	Columns: Per cent	Teliospores
Aug. 22, 1923	34	74	XXX	60	71	XX			
	46			85					
Aug. 29, 1923	112	86	XXX	138	69	XX-XXX,	124	78	XX-XXX,
	130			200		mostly XXX.	160		mostly XXX.
Sept. 5, 1923	26	19	X-XXX,	153	57	X-XXX,	19	6−	X-XX,
	140		mostly XX.	270		mostly high XX.	330		mostly X.
Sept. 12, 1923	103	52	X-XXX,	75	31	X-XX,	4	1+	X.
	198		mostly XX.	245		mostly XX.	301		
Sept. 19, 1923	52	49	X-XXX,	97	48	XX-XXX,	38	16−	X-XXX,
	107		mostly XX.	202		mostly XXX.	244		mostly XX
Sept. 26, 1923	9	10−	X-XX,	72	55	XX-XXX,	38	30	XX-XXX,
	93		mostly X.	130		mostly XXX.	125		mostly XXX.
Oct. 3, 1923	20	17	X-XX,	11	9	XX	11	8+	X-XX,
	119		mostly XX.	127			132		mostly X.
Oct. 10, 1923	1	1−	X	4	2+	X	2	2−	XX.
	110			175			101		
Oct. 17, 1923 [d e]	0	0	0	13	17	X	5	5−	X.
	3			77			111		
Oct. 24, 1923 [d e]	4	5	X	12	4+	X	6	2	X.
	76			285			293		
Oct. 31, 1923 [d e]	0	0	0	36	21	XX	0	0	0.
	168			170			250		

[d] No precooling.

[e] Tests at Bethel, Vt.

TABLE IV.—*Longevity of teliospores of Cronartium ribicola*—Continued

Date tested	Ribes species, place of collection, and date collected								
	R. nigrum (19), Wadhams, N. Y., Aug. 23, 1923			R. vulgare, (20), Caldwell, N. Y., Aug. 26, 1923			R. cynosbati (21), West Fort Ann, N. Y., Sept. 6, 1923		
	Germination								
	Columns		Teliospores	Columns		Teliospores	Columns		Teliospores
	Number	Per cent		Number	Per cent		Number	Per cent	
Aug. 29, 1922	315	89	Mostly XXX.	93	93	XXX [d]			
	355			100					
Sept. 5, 1923	215	68	X-XXX, mostly XX.	77	23	X			
	315			340					
Sept. 12, 1923	68	35	X-XXX, mostly XX.	61	21	X-XX, mostly X.	7	2+	X.
	196			288			285		
Sept. 19, 1923	78	27	X-XXX, mostly XX.	47	21	X-XX, mostly X.	4	2−	X.
	289			219			214		
Sept. 26, 1923	89	59	XXX	2	5−	X	19	16−	XX.
	150			41			121		
Oct. 3, 1923	105	66	XX-XXX, mostly XX.	1	1−	XX	1	1−	X.
	160			105			118		
Oct. 10, 1923	8	3+	X	0	0	0	1	1−	X.
	225			50			110		
Oct. 17, 1923 [d] [e]	75	61	XX	0	0	0	0	0	0.
	123			1			70		
Oct. 24, 1923 [d] [e]	87	50	XXX				0	0	0.
	173						230		
Oct. 31, 1923 [d] [e]	43	11−	XX						
	400								

[d] No precooling. [e] Tests at Bethel, Vt.

TABLE V.—*Summary of the longevity of teliospores of Cronartium ribicola*

Ribes species [a]	Number of days living	Per cent of columns germinating on last day tested	Amount of germination in column on last day tested	Number of days living in 1921 tests (16)
Americanum (13)	[b] 31	52	XX	-------
Americanum (17)	[b] 73	21	XX	[b] 59
Cynosbati (21)	35	0	0	-------
Cynosbati (6)	[b] 73	3	XX	59
Glandulosum (11)	[b] 47	6−	X	-------
Glandulosum (4)	69	0	0	-------
Glandulosum (14)	[b] 78	5−	X	-------
Nigrum (in house) (19)	[b] 49	1−	X	-------
Nigrum (in house) (1)	64	0	0	-------
Nigrum (from ground) (2)	57	0	0	-------
Nigrum (10)	[b] 47	3	X	-------
Nigrum (8)	66	0	0	-------
Nigrum (19)	[b] 70	11−	XX	-------
Nigrum (7)	[b] 87	21	XX	[b] 79
Odoratum (9)	44	0	0	-------
Odoratum (18)	63	0	0	[b] 59
Rotundifolium (5)	19	0	0	-------
Rotundifolium (15)	57	0	0	42
Triste (3)	41	2	X	-------
Triste (12)	54	0	0	-------
Vulgare (20)	39	0	0	-------
Vulgare (16)	70	0	0	-------

[a] Figure following species name is number of collection given in Table IV.
[b] Living when tests were discontinued.

It should be noted in Table IV that the tests of collections 3, 6, 10, 11, and 13 stopped abruptly before the limit of viability was reached. This was due to the lack of material which was scarce at the time of collection.

The germination tests given in Tables IV and V were made at 7-day intervals. Accordingly a lot of material which is said to have lived 35 days really lived a few days longer, since the teliospores actually died between tests. When there was enough material, a lot which gave no germination in a test was retested twice more to insure accuracy.

When the tests were concluded the teliospores on *Ribes americanum* were germinating rather strongly, although they had weakened perceptibly. Those on *R. glandulosum* were still germinating, but were rather weak and evidently approaching the limit of their viability. As in 1921 (*16*), some of the teliospores from *R. nigrum* were germinating strongly, and, considering their age, relatively abundantly. In view of the results obtained in 1921, there seems to be no good reason for believing that these strongly germinating teliospores would lose their viability before winter set in.

On the other hand, three lots of teliospores from *Ribes nigrum* had died about the first of October. It appears that the teliospores from *R. rotundifolium* may lose their viability rather quickly. However, those from the latter species, collected August 4, were borne on leaves which had both surfaces completely coated with dew at the time of collection. Partial germination had taken place, and the viability of the remaining teliospores was unusually low, as shown by tests made immediately after collection. The telial columns collected August 3 on leaves of *R. triste* and *R. glandulosum* were probably quite old, because of a local drought which stopped telial production and germination (*13*).

Teliospores on naturally fallen leaves of *Ribes nigrum*, collected from the ground, lived 57 days when kept out doors. This indicates that infected leaves, even when lying on the ground, may be sources of pine infection for a prolonged period. It is well known that such leaves may be blown for some distance (*17*). These facts complicate the making of direct field observations upon the distance that sporidia may travel and cause pine infections. But such field observations are valuable in determining how far infection may extend, regardless of the manner, from Ribes to pines.

Teliospores on *Ribes nigrum*, collected August 23 and stored indoors, lived 49 days, while part of the same material kept out doors exposed to wind and rain gave 11 per cent germination after 70 days. Another lot of material from *R. nigrum*, collected August 1 and kept indoors, gave germination after 64 days.

Early in January, 1924, teliospores from Ribes leaves which had been kept dry in folders were tested for viability by the floating method. The collections were as follows: 2 made in 1915; 3 in 1916; 4 in 1919; 3 in 1920; 2 in 1921; 3 in 1922; 5 in 1923; and 4 in 1924 (fresh material from the greenhouse). In most collections, persisting old promycelia were observed in some abundance, but no new germination of teliospores was observed after 24 and 48 hours, except in the 1924 material, in which the germination was very good within 24 hours.

LONGEVITY OF UREDOSPORES

During the summer of 1923 no material was collected primarily for the purpose of studying the longevity of uredospores. However, in the course of the studies to determine the longevity of teliospores it was found that practically all of the leaves which had produced telial columns had also a generous number of uredospores present. It is often found that leaves apparently bearing only telia also have fresh uredospores borne around the bases of the telia. These probably are the source of the new uredinial sori which often appear on new leaves late in the season. With the above material, in each test in which teliospores were germinated, the germination of the accompanying uredospores was also noted. This means that observations were made only on such uredospores as were floating on the glasses of water together with the telial columns.

Tests of longevity of uredospores of other rusts have been made by a number of workers (*12*). The results with uredospores of *Cronartium ribicola* range from 7 to 270 days (*17*) under various conditions. Table VI gives the number of days which the uredospores from the various Ribes species remained living after telial columns had formed and after the leaves had been picked. From Table VI it will be seen that no germination of uredospores was accomplished on *Ribes triste*, while on *R. nigrum* the uredospores lived as long as 59 days. On leaves collected from the ground the uredospores lived 50 days, and on material kept in the house they remained viable 36 days; hence the length of time that uredospores can live on leaves after teliospores have been formed varied from 0 to 59 days, in these tests of the writers. The uredospores, as explained under discussion of Table IV, actually lived slightly longer than these figures indicate.

TABLE VI.—*Longevity of uredospores of Cronartium ribicola*

Ribes species *	Date of collection, 1923	Last date alive, 1923	Number of days living	Ribes species *	Date of collection, 1923	Last date alive, 1923	Number of days living
Americanum (13)	Aug. 13	Sept. 5	24	Nigrum (10)	Aug. 11	0	0
Americanum (17)	Aug. 20	Oct. 3	45	Nigrum (19)	Aug. 23	Oct. 10	49
Cynosbati (6)	Aug. 6	Aug. 8	3	Odoratum (9)	Aug. 7	Aug. 29	23
Cynosbati (21)	Sept. 6	Sept. 9	4	Odoratum (18)	Aug. 23	Oct. 3	42
Glandulosum (4)	Aug. 3	Aug. 29	27	Rotundifolium (5)	Aug. 4	0	0
Glandulosum (11)	Aug. 11	do	19	Rotundifolium (15)	Aug. 15	Aug. 20	6
Glandulosum (14)	Aug. 15	Aug. 20	6	Triste (3)	Aug. 3	0	0
Nigrum (1)	Aug. 1	Sept. 5	36	Triste (12)	Aug. 11	0	0
Nigrum (2)	do	Sept. 19	50	Vulgare (16)	Aug. 16	Aug. 20	5
Nigrum (7)	Aug. 6	Oct. 3	59	Vulgare (20)	Aug. 26	Sept. 12	18
Nigrum (8)	do	Aug. 15	10				

* Figure following species name is the number of collection given in Table IV.

The viability of uredospores in old material was tested at the same time (January, 1924) and from the same material as here reported for teliospores. Examination of each of several collections showed a very few uredospores with persisting old germ tubes. Spores which had been floated on water had a few similar persisting old tubes but no new ones. The fresh uredospores germinated abundantly. M. W. Taylor found similar persisting germ tubes among old aeciospores of this fungus. In making germination tests of old spores, it is necessary to examine first for these persisting old germ tubes.

SUMMARY

During the summer of 1923 telia from eight Ribes hosts were tested for longevity. Under outdoor conditions their longevity varied from 19 days for one collection of *Ribes rotundifolium* to 87 days for *R. nigrum*, which still germinated well at the end of the experiments.

The "floating" method of germination was much superior to the damp-chamber method.

Precooling per se did not seem to stimulate germination of teliospores markedly. Tests are needed to prove whether decided alternations in temperatures are not the stimulating factors.

The period of germination of teliospores increases with their age, whether kept dry indoors or outdoors exposed to the weather.

Uredospores accompanying the teliospores remained viable for a maximum period of 59 days under the conditions of these experiments.

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THE CRITICAL TEMPERATURE FOR INFECTION OF THE POTATO SEED PIECE BY FUSARIUM OXYSPORUM [1]

By H. G. MacMillan, *Pathologist*, and G. A. Meckstroth, *Assistant Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The infection of the potato (*Solanum tuberosum*) seed piece by *Fusarium oxysporum* Schlect. commonly results in much disease and consequent economic loss in the western part of the United States. Of the factors influencing the development of this disease one of the most important is temperature, for it is an element of the environment to which both host and parasite respond. The effects of other elements are altered by its changes. The critical temperature of infection—that is, the point below which parasitism fails to occur, and above which damage to it develops—is the point of departure for studies of wilting, rotting, and other manifestations of infection.

REVIEW OF LITERATURE

In 1919 MacMillan (*8*) [2] pointed out the fact that temperature is vital so far as infection of the potato seed piece by *Fusarium oxysporum* is concerned. Other Fusaria and different host plants have received the attention of a number of workers. Gilman (*3*) determined 17° C. to be the critical temperature of infection of cabbage by *F. conglutinans*. Tisdale (*9*) found flax to be infected by *F. lini* at 15° or higher. Clayton (*1*) determined 22° as approximately the critical temperature for infection of the tomato by *F. lycopersici*. Johnson and Hartman (*6*) reported that the root-rot disease of tobacco less marked below 15°, and that a number of other soil factors influence the development of it. Goss (*5*) found that the wilt of potatoes caused by *F. oxysporum* did not develop below 18°. In the determination of these temperatures one type of apparatus was used by all, the so-called Wisconsin temperature tank, described by Jones (*7*).

Under practical conditions no constant temperature of the soil is to be expected. Dickson (*2*) states that the mean of a fluctuating soil temperature develops the same condition as a constant temperature equal to the mean. But for the exact determination of a critical temperature point as constant a temperature as possible is desired. Goss (*4*) in a controlled temperature study of Fusaria rots maintained the temperatures with a variation of only 2° C. Other workers have allowed variations of these small amounts, inevitable from the nature and duration of the experiment.

[1] Received for publication Mar. 21, 1925; issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 921.

APPARATUS AND METHODS

The apparatus used for maintaining the temperatures of the experiments herein reported was an adaptation of the Wisconsin tank method. Essentially, it consisted of a series of water baths for small soil cans. A large wooden bin about 4 by 20 feet was built in the greenhouse, and 12 round, galvanized tanks, each 16 inches in diameter and 26 inches deep, were so arranged in the bin as to allow a maximum amount of space around each tank. The bottom of the bin and the spaces between the sides of it and the tanks were well packed with sawdust and straw as a means of partial insulation. A ½-inch brass tube was soldered into the side of each tank at the bottom, and a rubber tube attached which reached to the outside of the bin, where it was closed with a spring clamp. The bin and tanks were covered with boards closely fitted. Over each tank three holes 5 inches in diameter were cut, the centers being equally spaced on a circle the radius of which was 3.5 inches, and the center of which was approximately the center of the tank. At the center and between the three large holes a small hole was made to receive a thermometer. Thirty-six galvanized-iron cans, each 5 by 12 inches, were prepared with a wire lip at the top, so that they would not fall through the hole in the cover above the tank.

The method of preparation of the soil and seed potatoes for use in these cans was simple. A uniform quantity of a suitable potato soil was prepared by much working and shoveling, and brought to a uniform moisture content of approximately 18 per cent. The soil and cans were sterilized by steaming. The soil was cooled and mixed again. Potatoes of the Early Ohio variety were used. They were of uniform size, each weighing about 4 ounces. The tubers were immersed in a 1 to 1,000 solution of mercuric chloride for one hour, then rinsed with sterile water. Following this, they were cut once lengthwise with a sterile knife, inoculated, and planted immediately. One can of the three for each tank was used as a control. The surface of the control seed piece was treated with 2 cubic centimeters of sterile distilled water. The seed pieces for infection were inoculated with 2 cubic centimeters of a heavy spore suspension of *Fusarium oxysporum* in sterile distilled water. Six seed pieces were planted in each can in two layers, three in each layer. The top layer came within 4 inches of the soil surface, and was about 5 inches below the surface of the water in the tank. The cans were brought to uniform weight by the addition of soil. They were then placed in the holes in the cover of the tanks. The tanks had been previously filled to the top with water and adjusted to the desired temperature.

No automatic device was used to regulate or control the temperatures. The tanks were under observation continuously for the duration of the experiment. The thermometers used were carefully calibrated, and corrections compensated for in the readings. The temperature of the water was raised by introducing an electric immersion heater, and lowered by the addition of cold water, with the removal of an equal volume at the bottom. Before and after each change the water was thoroughly agitated with a 4-inch propeller on a long shaft turned by a hand drill. In order to add water and insert the propeller a can had to be lifted. They were lifted in rotation. Thermometers in them detected no change of tempera-

ture in this process, since the time involved was usually less than a minute. The 12 tanks were paired, and six different temperatures maintained. Comparisons between the temperature of the tank and the temperature of the soil in the cans at the depth at which the potatoes were showed them to be the same.

THE PRELIMINARY EXPERIMENT

The temperatures for the preliminary experiment were 14°, 16°, 18°, 20°, 22°, and 24° C. Readings were recorded every 2 hours, although corrections were frequently made oftener. The first experiment ran from 6 p. m. February 16, 1922, to 6 p. m. February 26, 1922, a period of 240 hours. An effort was made to maintain the greenhouse temperature at about 18° C., but it rose during the day and frequently fell during the night. Even with care there were deviations from the desired temperatures, but rarely more than two or three tenths of a degree. The tank at the high-temperature end of the bin came too near the heating pipes, and the temperature was kept down with difficulty.

The average deviation for the period of the experiment is given in Table I.

TABLE I.—*The temperature which it was desired to maintain in each tank in the preliminary experiment, together with the average deviation for the experimental period, expressed in degrees centigrade*

Desired temperature	14.0	16.0	18.0	20.0	22.0	24.0
Average deviation:						
First tank	+0.049	+0.013	−0.002	−0.003	−0.020	−0.010
Second tank	+0.005	−0.049	+0.002	+0.052	+0.027	+0.142

At the termination of the experiment the cans were opened and the seed pieces examined for evidence of infection. The seed pieces in the control cans were clean, the cut surface bright. To all appearances they were about as when planted. The seed pieces inoculated with *Fusarium oxysporum* showed varying degrees of infection, and all of them showed evidence of contact with the fungus. At 24° C. all were well infected, the decay varying in depth from 2 millimeters to 1.5 centimeters, the epidermis was shrunken, and the flesh discolored. At 22° all were well infected, though the infection was not as far advanced as at 24°. At 20° and 18° all pieces were infected in much the same manner as at 22°, but with less penetration into the flesh of the seed piece. At 16° infection was more moderate, all pieces were infected, but decay penetrated only to a depth of about 2 millimeters. At 14° infection was not apparent. The inoculated surface was dark, the tissue was slightly browned just under the surface, and there was no shrinking at the edges. Upon reisolating the fungus it was secured in pure culture from all of the seed pieces maintained at 16° or higher, and from two seed pieces held at 14°.

FINAL DETERMINATION OF CRITICAL TEMPERATURE FOR INFECTION

In order to more carefully determine the temperature at which infection occurs the experiment was repeated, a restricted range of temperatures being used. The cans were sterilized, the soil was pre-

pared as before, potato seed pieces from the same lot of potatoes were prepared in the manner described above, and the thermometers were recalibrated. The tanks were adjusted by pairs to temperatures of 13°, 13.5°, 14°, 14.5°, 15°, and 15.5° C. The duration of the experiment was 300 hours, beginning at 6 p. m. March 9, 1922, and ending at 6 a. m. March 22, 1922. Readings and adjustments were made every hour. The average deviations from the desired temperatures are given in Table II.

TABLE II.—*The temperature which it was desired to maintain in each tank in the final experiment, together with the average deviation for the experimental period, expressed in degrees centigrade*

Desired temperature	13.0	13.5	14.0	14.5	15.0	15.5
Average deviation:						
First tank	+0.0133	+0.008	−0.0003	−0.0073	−0.0053	−0.0012
Second tank	+0.004	+0.0003	−0.0013	−0.0073	+0.0046	+0.0033

At the termination of the experiment the cans were lifted, and the seed pieces removed and examined for evidence of infection. All of the controls appeared distinctly different from the inoculated seed pieces held at the same temperature. The cut surfaces were clean and bright as if almost freshly cut. The inoculated pieces held at 13° were more heavily calloused than the controls, and appeared a trifle darker, but there was no evidence of infection in any case. The buds of the seed pieces had germinated normally. Of the seed pieces inoculated with *Fusarium oxysporum*, none held below a temperature of 14.5° C. appeared to be infected. They seemed to be a little more heavily calloused, and very slightly shrunken about the edges. At 14.5° and above the seed pieces were more or less shrunken at the edges, and the cut surfaces had developed a mottled appearance, frequently characteristic in this infection. The reisolation of the causal fungus from the tissue below the surface was depended upon to show whether or not infection had occurred. Mere examination was insufficient. The tubers were broken open toward the cut surface, and fragments of tissue below the surface, but not including it, were transferred to poured plates of potato agar. Where a fusarium developed it was transferred to suitable tube media and identified. From the seed pieces held at 13° and 13.5° no fungus developed. Of those held at 14°, six pieces, or 25 per cent, yielded *F. oxysporum* in pure culture. At 14.5°, 15°, and 15.5° all seed pieces yielded *F. oxysporum* in pure culture.

The critical temperature for infection of the potato seed piece was found to be approximately 14° C. under conditions such as prevailed during the course of these experiments.

DISCUSSION

The conditions under which the experiments were performed were approximately normal, so far as the soil was concerned. Because infection occurred at 14° C. it must not be assumed that wilt or death of the plant would have taken place had the experiment lasted long enough to have produced plants. Vegetative activity offers resistance to the advance of seed-piece rot. Other factors, such as vigor of seed and aeration, commonly prevent or retard seed-piece

decay, even when the soil temperature is well suited to it, and the fungus is present. As has been pointed out before (*8*), infection appears to be universal in certain sections of the West, and there is no reason for withdrawing this statement. Decay does not necessarily follow infection at once, and may not over an extended period, but when the proper conditions arise epidemics develop. A low initial infection temperature assists the disease to appear in epidemic form.

CONCLUSION

Under approximately normal conditions and at constant temperatures, seed pieces from potatoes of only moderate resistance are infected by *Fusarium oxysporum* at approximately 14° C., but not at lower temperatures.

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SOME POSSIBLE ERRORS IN THE USE OF CURVES[1]

By DONALD BRUCE

Silviculturist, Forest Service, United States Department of Agriculture

Although the analysis of data by means of graphs and curves has been one of the most common procedures in forestry, little investigation apparently has been made of the limitations of the method or of the seriousness of possible errors which may result from ignorance or disregard thereof. The basic idea of plotting measurements on coordinate paper and of fitting curves thereto is derived from analytical geometry, and there has been a tendency to assume that any of the processes of the algebraic basis for this branch of mathematical science are equally permissible in the case of empirical curves. Pure mathematics, however, assumes exact equations between the variables under consideration, and these variables are therefore perfectly correlated. Empirical data are never perfectly correlated and are usually (at least in the case of forestry) very loosely correlated. As the degree of correlation becomes less, it can be shown that certain mathematical processes become increasingly inapplicable until in many instances gross errors are introduced by their use.

Probably the best way to make this possibility of error clear is to take a single data series and subject it to apparently plausible transformations, comparing the results in each case with those obtained by a more direct handling. The material selected is taken from measurements of a series of 524 second-growth long-leaf pine trees and, for simplicity, is limited to the three measurements: Diameter (breast high), height, and volume in cubic feet. This is a case in which the variables are comparatively closely correlated and in which, therefore, no abnormally serious discrepancies should occur. Few forestry problems afford better material, and if any important errors are discovered in this instance, difficulties at least as great may be expected wherever parallel processes are used.

Let us consider first the possibility of interchanging the independent and the dependent variables. Texts in forest mensuration have warned against this, but without sufficiently emphasizing the kind and degree of error involved therein. At first glance, it seems quite obvious that if a table or curve showing average heights of trees of given diameters is available, then the average diameters of trees of given heights should be obtainable therefrom. To do so would be analogous to transforming an algebraic equation such as $y=ax+b$ into $x=\frac{y-b}{a}$, and it is probably because of the correctness of such an algebraic transformation that the temptation to perform a similar process with empirical curves is so strong. But let us see the result.

[1] Received for publication Jan. 29, 1925; issued January, 1926.

In Figures 1 and 2 the data series just described are plotted, first with diameter at breast height and then with height as the independent variable. In Figure 1 each cross represents the average

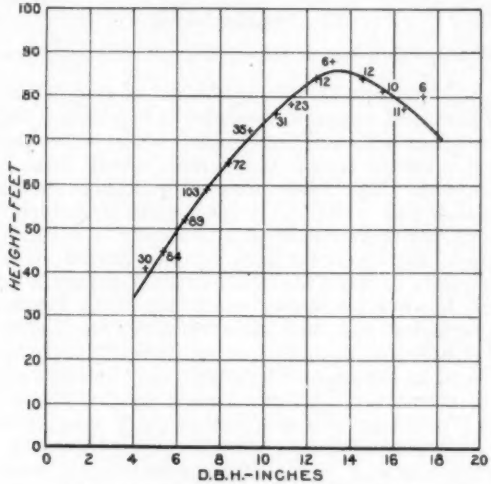

FIG. 1.—Curve of height over diameter breast high

height of all the trees in a single 1-inch diameter class, the adjacent figure indicating the number of trees involved in each average. In Figure 2, similarly, each cross represents the average diameter at

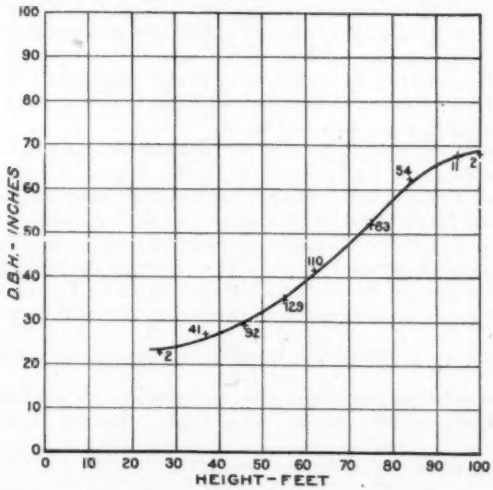

FIG. 2.—Curve of diameter breast high over height

breast height for all the trees in a single 10-foot height class. Each curve is quite strongly defined. Some surprise may be occasioned by the fact that the first of these curves falls after passing 13 inches

in diameter, but this is readily explainable on the ground that the trees of largest diameter were undoubtedly those which were standing isolated from their neighbors, and which were shorter than those growing in denser stands. It should be noted, also, that curve 2 does not necessarily originate from the point 4.5 – 0, because it is neither a growth curve nor (what is very similar) a relationship curve for a strictly selection stand.

If the two curves are carefully compared it will at once be seen that the one is not merely a reversal of the other. The extent of the difference is best brought out by Figure 3. In this the curve of Figure 1 is replotted, while that of Figure 2 (the broken line) is turned on its side so that its axes coincide with those of curve 1. Through the central part of the curves the errors are relatively small, but the upper end is startlingly different, both in position, trend, and range

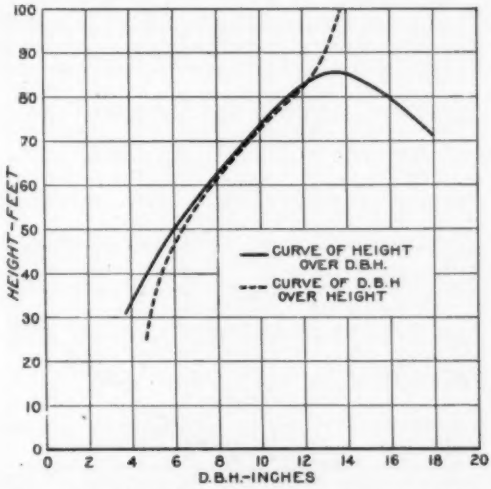

FIG. 3.—Comparison of curves of height over diameter, and of diameter over height, for same data

of values. It is clear that to reverse either curve and use it as a substitute for the other will result in errors which may be very serious. The approximate coincidence of the two curves for the central half of their range is an indication of quite high correlation, but in spite of this the distortion at the upper end is so great that the most interesting fact brought out by the upper part of curve 1 is completely obliterated.

A second type of transformation which seems equally plausible (and on which the texts are silent, if not actually misleading) suggests itself in a case such as the following. Let us assume we have at hand a curve of height over diameter at breast height (such as fig. 1) and one of volume over height (such as fig. 4). Let us also assume that we wish a curve showing the relation between volume and diameter. Apparently we can obtain this curve directly from the two already drawn without having to turn once more to the original data, a process which would involve a considerable amount of labor in

regrouping and averaging. We have once more an apparent precedent in algebra, for if we have equations between x and z and y and z such as $z=ax+b$ and $y=cz+d$, we may with perfect propriety substitute the value of z from the first equation in the second and obtain, as the equation between x and y,

$$y=c\ (ax+b)+d=acx+bc+d$$

But again, let us examine the actual result with our empirical data.

The curve in Figure 5 is thus worked out. For example, from Figure 1 we find that the average height of 4-inch trees is 34 feet. From Figure 4 we find that the average volume of 34-foot trees is 1.6 cubic feet. We may therefore plot 1.6 cubic feet as the average volume of 4-inch trees. In a similar way we may find values for volumes corresponding to as many successive values for diameter as

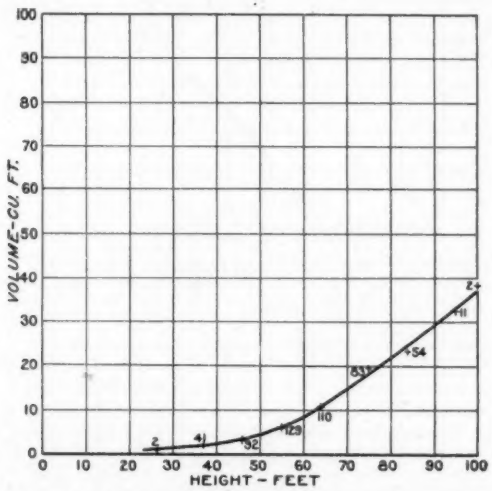

FIG. 4.—Curve of volume over height, for same data

we please. Since the values are derived from two curves they will themselves inevitably fall on a smooth curve.

The best test of the accuracy of this curve is to see how well it fits the data when the latter are handled in the straightforward manner. The same material has therefore been sorted into 1-inch diameter classes and the average value for each class obtained. The resulting values are plotted as small crosses in Figure 5. It is clear that the derived curve is entirely inadequate. Even at the lower end the percentage differences between the plots and the curve are very considerable (over 30 per cent), while at the upper end the discrepancies in both position and trend are gross.

Nor is this accidental. With three variables under consideration six curves are possible (height over diameter, diameter over height, height over volume, volume over height, volume over diameter, diameter over volume). All six of these have been drawn both directly from the data and by the indirect method just described.

In every instance large discrepancies were found, which are so similar in character to those illustrated in Figure 5 that to present them here would introduce no new element.

In both the instances which have been described the way in which the erroneous results are obtained can best be understood by actually working out an example, and carefully noting how the different individual values combine with other values and exert their influence on different parts of the curve. In the last instance, for example, it will readily be seen by inspecting the curves that for any given diameter the particular trees which determine the average height (curve 1) are for the most part entirely different from those which determine the corresponding average volume. The possibility that diversity in manner of grouping is the prime cause of the difficulties encountered suggests an alternative procedure.

Suppose measurements are sorted by height class, and that the average diameter at breast height and average volume for each class are determined. By plotting these average volumes over these average diameters the curve which has been sought can apparently be

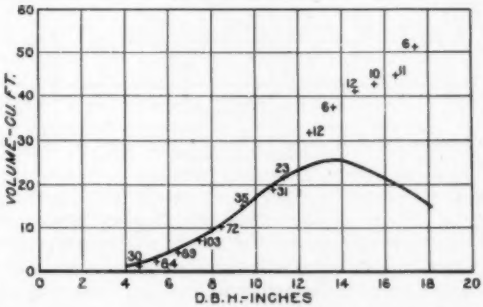

FIG. 5.—Curve of volume over diameter, obtained indirectly from curves of Figures 4 and 1, compared with actual averages

obtained in still a third way. Furthermore, it is immaterial whether actual averages be used or whether advantage be taken of the fact that curves have already been drawn for each of these variables over height by using corresponding curve values. The algebraic analogy would be almost identical with that just described.

For example, we may find from Figure 4 that the average volume of 30-foot trees is 1.3 cubic feet, and from Figure 2 that the average diameter at breast height for 30-foot trees is 4.8 inches. We may then plot 1.3 over 4.8. Repeating the process for other height classes, we produce the volume-diameter curve illustrated in Figure 6. When the actual average values are plotted, however, as has been done in the figure, this curve in turn is seen to be a very poor expression of the data. Particularly noticeable is the fact that the curve covers only about two-thirds of the range of the points and gives no values at all for the higher diameters. In the present instance the result of this last process is perhaps less erroneous than that previously illustrated (fig. 5); but when similar procedures were tried with the other five possible curves, it was found that this superiority was accidental, and that in general there was little or no choice.

It appears, then, that a curve based on empirical data can neither be used backwards nor combined with other curves, without serious liability of gross errors. It also appears that material should not be sorted for averaging in any way except on the basis of the independent variable. The seriousness of the errors depend largely on the degree of correlation between the variables. Yet in the literature of forestry may be found innumerable examples in which analogous processes are involved. The first error is more generally recognized and more generally avoided, although tree taper curves, for example, are still quite generally advocated for obtaining average heights to given

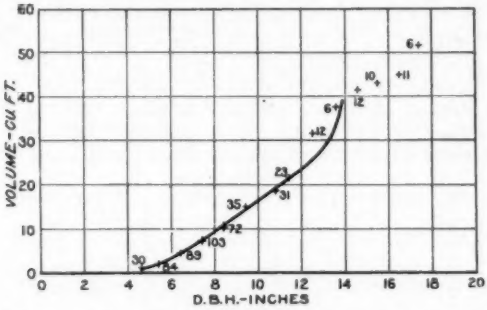

FIG. 6.—Curve of volume over diameter, obtained indirectly from curves of Figures 4 and 2, compared with actual averages

diameter limits, whereas they give accurately only average diameters at given heights. The second error is probably more common than the third. It is exemplified in the study of uneven-aged stands by treating diameter classes as if they were age classes, when once the relation between age and diameter is worked out. In this case the correlation is obviously quite low, and one can but surmise how inaccurate the results thus obtained may be. A determination of the coefficient or index of correlation in such cases may suggest how great the dangers in the indirect treatment are, but reliability can only be obtained by the simple direct method, laborious though it may prove to be.

A PRELIMINARY STUDY OF THE GROWTH OF NOBLE FIR[1]

By E. J. Hanzlik,

Forest Inspector, Forest Service, United States Department of Agriculture

INTRODUCTION

Noble fir (*Abies nobilis*) is one of the main components of the forest in the upper-slope type on the west slope of the Cascade Range in Oregon and Washington. It rarely occurs in pure stands. In the lower part of its range, it is usually found in mixture with Douglas fir (*Pseudotsuga taxifolia*), western hemlock (*Tsuga heterophylla*), and amabilis fir (*Abies amabilis*). In its upper limits it is associated with western white pine (*Pinus monticola*), amabilis fir, and yellow cypress (*Chamaecyparis nootkatensis*) in Washington and northern Oregon, and with white fir (*Abies concolor*), white pine, and hemlock in southern Oregon. It also occurs in the Olympic Mountains of Washington, and is found as a rare tree in the Coast Ranges of Oregon.

The altitudinal limits of the noble fir lie between 3,000 feet and 5,000 feet, occurring at this higher point only in the southern part of its range. In northern Washington its upper limit is between 4,000 and 4,500 feet. Below 3,000 feet it occurs only as a rare, scattered individual in mixture with the Douglas fir.

The data used in the present study were obtained in connection with a general study of western hemlock upon the logging operation of the Bridal Veil Lumber Co. near Palmer, Oreg., on the west slope of Larch Mountain (T. 1 N., R. 6 E., W. M.)

The area involved presented a west to southwest aspect on a gentle to moderate slope, at an elevation of from 3,000 feet to 3,500 feet. The topography is relatively smooth, and the drainage is very good, the area being cut into gentle contours by a number of small streams.

The soil consists of a clayey to a sandy loam, 6 to 18 inches deep, with a moderate depth of humus and decaying vegetable matter on the ground. The subsoil is composed of rock and gravel, none of the rock outcropping to the surface.

The climatic conditions are such as to be quite favorable to tree growth. The temperature does not vary greatly during the different seasons. The summers are fairly cool, with an abundance of fog and mist; while the winters, although having an excessive snowfall, are not subject to extremely low temperatures. The precipitation ranges from 80 to 90 inches per annum. For the best development of noble fir, a comparatively humid climate and a fresh, deep, porous soil are required. Although this locality is a quality III site of the lower slope type for Douglas fir, it is the best quality upon which noble fir grows, as this tree does not form any stands at lower elevations.

The stand represents a mature forest composed of noble fir, Douglas fir, and western hemlock, with a scattering of amabilis fir. This

[1] Received for publication Dec. 23, 1924; issued January, 1926.

last-named species becomes more abundant with an increase in elevation, taking the place of hemlock and Douglas fir, these species dropping out. Noble fir and Douglas fir form the dominant crown classes, hemlock occurring mainly as an understory. In point of numbers, hemlock forms about 60 per cent of the stand, noble fir and Douglas fir about 20 per cent each; according to volume, however, noble fir forms 46 per cent, Douglas fir 24 per cent, and hemlock about 30 per cent. The crown canopy varies from 0.8 to 0.9 in point of density for the upper story, which, coupled with the canopy of the suppressed hemlock, casts a rather dense shade. This is probably the main reason for the almost total lack of underbrush, except where the crown has been opened owing to windfall or death of the dominant trees.

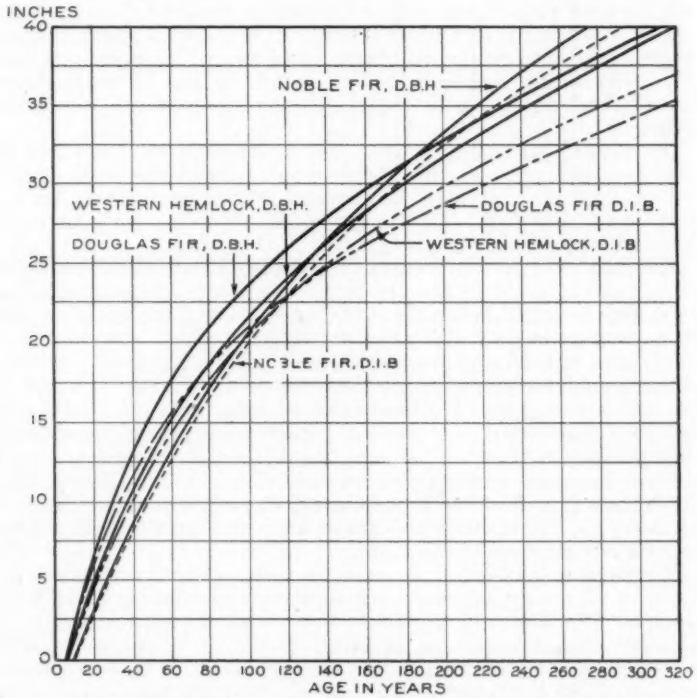

FIG 1.—Diameter-growth curves for noble fir, Douglas fir, and western hemlock

THE TREE AND ITS MANAGEMENT

Noble fir in this locality forms a tall, straight, cylindrical bole with a comparatively small and open crown. The trunk prunes itself readily of side branches, indicating the rather intolerant nature of the tree; the length of the crown being only about one-third to one-fourth of the total height. Trees were found up to 6 feet in diameter at breast height, attaining a maximum height of about 215 feet. The average tree, however, has a diameter of a little more than 4 feet, and an average height of close to 200 feet.

Noble fir is less tolerant than either western hemlock or amabilis fir, its light requirements being about the same as those of Douglas fir. Seedlings are seldom found in the shade of older stands, the undergrowth usually consisting of stunted hemlock and amabilis fir.

Noble fir seed will germinate both upon bare mineral soil and on duff, preferably the latter. It requires rather moist soil conditions for best results. Reproduction takes place only in openings, such as are caused by fire or lumbering.

Noble fir is probably best adapted to a mixed type of forest where it is able to maintain a dominant position in the stand, such as when in mixture with Douglas fir and hemlock, or amabilis fir and hemlock. Since it does not reproduce itself under shade, clear cutting in groups or strips is apparently necessary to obtain satisfactory natural reproduction. In the present mature stands, in order to accomplish natural restocking, cutting should take place in the spring following a good seed year. This will insure a crop of seed on the ground which should find favorable conditions for germination after the old stand has been cut. Fire should be kept out of the cut-over area. On the upper slopes, where for protective reasons clear-cutting can not be followed, the selection system is applicable, in which rather heavy cuttings should be made in order to open up the stand sufficiently for noble fir to take hold. Due to its superior qualities, noble fir should be favored more than any other species, with the exception of western white pine, in the upper portions of the lower-slope and in the lower portions of the upper-slope type.

GROWTH

It is probably in direct competition with a rather intolerant species such as Douglas fir that noble fir attains its best growth, both in diameter and in height.

Figure 1 illustrates graphically the diameter growth of noble fir, Douglas fir, and western hemlock in a mature stand, where the trees of all species averaged about 400 years of age. The results are for all the dominant trees on the tract.

Table I gives the average rate of diameter growth and height growth of noble fir in a mixed stand of Douglas fir and western hemlock at a elevation of from 3,000 to 3,500 feet on the west slope of the Cascade Mountains in northern Oregon.

TABLE I.—*Average rate of diameter and height growth of noble fir, in mixed stand, on the west slope of Larch Mountain, Oreg.*

Age	Average diameter at breast height	Average annual diameter growth in each decade	Average total height	Average annual height growth in each decade	Age	Average diameter at breast height	Average annual diameter growth in each decade	Average total height	Average annual height growth in each decade
Years	*Inches*	*Inches*	*Feet*	*Feet*	*Years*	*Inches*	*Inches*	*Feet*	*Feet*
10	0.0		4.0		130	25.0	0.14	116.1	0.51
20	3.1	0.31	12.0	0.80	140	26.3	.13	120.9	.48
30	6.0	.39	23.5	1.15	150	27.6	.13	125.4	.45
40	8.7	.27	38.4	1.49	160	28.8	.12	129.7	.43
50	11.1	.24	53.0	1.46	170	30.0	.12	133.8	.41
60	13.3	.22	65.5	1.25	180	31.1	.11	137.8	.40
70	15.3	.20	76.2	1.07	190	32.2	.11	141.7	.39
80	17.1	.18	85.2	.90	200	33.2	.10	145.5	.38
90	18.8	.17	92.8	.76	250	37.9	.09	162.2	.31
100	20.5	.17	99.5	.67	300	42.1	.08	175.8	.26
110	22.1	.16	105.5	.60	350	45.9	.07	187.3	.21
120	23.6	.15	111.0	.55	400	49.4	.07	196.3	.16

Table II gives the seedling height growth of noble fir grown under average conditions in the same locality as indicated in Table I.

Table III gives the average number of years required for noble fir seedlings to attain various heights.

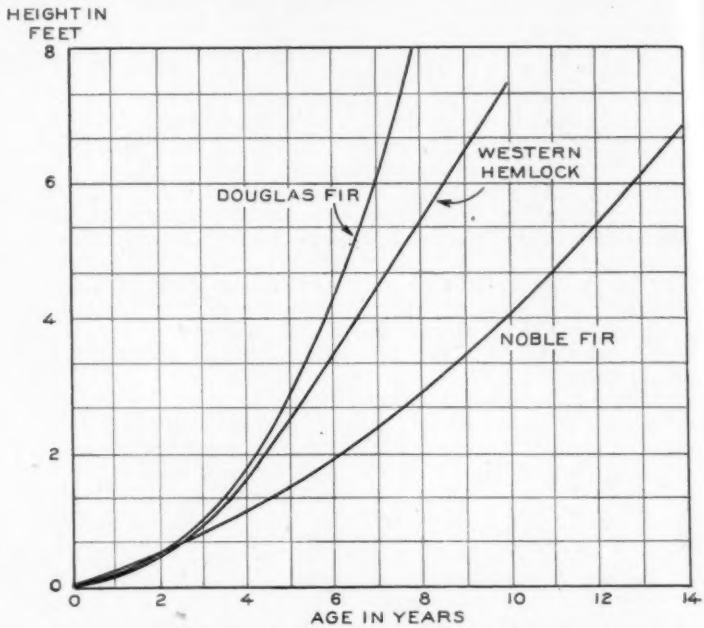

FIG. 2.—Height-growth curves for seedlings of noble fir, Douglas fir, and western hemlock.

Figure 2 illustrates graphically the seedling height growth of noble fir, Douglas fir, and western hemlock, in western Washington and Oregon.

TABLE II.—*Seedling height growth of noble fir* [a]

Age	Height	Current annual growth	Age	Height	Current annual growth
Years	*Feet*	*Feet*	*Years*	*Feet*	*Feet*
1			9	3.45	.55
2			10	4.04	.59
3	0.84	0.28	11	4.69	.65
4	1.16	.32	12	5.38	.69
5	1.53	.37	13	6.12	.74
6	1.94	.41	14	6.88	.76
7	2.39	.45	15	7.62	.74
8	2.90	.51	16	8.32	.70

[a] Based on 282 measurements.

The seedling height growth of noble fir is slower than that of either Douglas fir or western hemlock. To attain breast height, 4.5 feet above the ground, it takes noble fir an average of 11 years, open-grown western hemlock 7 years, and Douglas fir 6 years.[2]

[2] MUNGER, T. T. THE GROWTH AND MANAGEMENT OF DOUGLAS FIR IN THE PACIFIC NORTHWEST. U. S. Dept. Agr., Forest Serv. Circ. 175, 27 p., illus. 1911.

TABLE III.—*Average number of years required for noble fir seedlings to attain various heights, Larch Mountain, Oreg., 3,300 feet elevation*

Height	Age	Height	Age	Height	Age	Height	Age
Feet	*Years*	*Feet*	*Years*	*Feet*	*Years*	*Feet*	*Years*
0.5	2	2.5	7	4.5	11	6.5	14
1.0	3	3.0	8	5.0	11	7.0	14
1.5	5	3.5	9	5.5	12	7.5	15
2.0	6	4.0	10	6.0	13	8.0	16

YIELD

Table IV gives the average contents per acre of standing trees of different species, no reduction being made for defect or breakage, for two plots differing essentially in the number of dominant trees forming the stand. Table V gives the average contents for 17¾ acres.

TABLE IV.—*Average number of trees, average diameter, and average volume, by tree classes and species* [a]

Plot and species	Dominant trees per acre	Diameter of average dominants	Total volume of dominants per acre	Suppressed trees 12 inches and more in diameter per acre	Volume of suppressed trees per acre	Total trees per acre with breast-height diameter of 12 inches or more	Total volume of all trees per acre
Plot 1—4 acres:	*Number*	*Inches*	*Bd. ft.*	*Number*	*Bd. ft.*	*Number*	*Bd. ft.*
Noble fir	8.50	45.6	51,887	1.00	1,125	9.50	53,012
Western hemlock	4.25	42.1	16,892	20.25	13,922	24.50	30,814
Douglas fir	12.25	42.6	41,111	1.50	1,892	13.75	43,003
Red cedar	.75	48.8	2,327	.75	384	1.50	2,711
Total	25.75		112,217	23.50	17,323	49.25	129,540
Average		43.7					
Plot 2—3 acres.							
Noble fir	8.33	55.0	79,667	1.33	536	9.66	80,203
Western hemlock	3.33	44.1	14,821	30.66	39,594	34.00	54,415
Douglas fir	4.00	48.5	19,508	.66	1,127	4.66	20,635
Amabilis fir				.33	267	.33	267
Total	15.66		113,906	33.00	41,524	48.65	155,520
Average		51.2					

[a] Under "dominants" are included both dominants and codominants in the principal stand.

TABLE V.—*Average yield per acre of mature noble fir, Douglas fir, and western hemlock, on Larch Mountain, Oreg.* [a]

Species	Trees per acre	Basal area	Diameter of average tree	Yield per acre
	Number	*Sq. ft.*	*Inches*	*Bd. ft.*
Noble fir	10	127	47.6	69,415
Douglas fir	7	66	42.0	22,208
Western hemlock	40	146	25.9	49,427
All species	57	339	30.4	141,110

[a] Averages for 17¾ acres. Data are for trees 12 inches or more in diameter at breast height. Noble fir is utilized to a top diameter of 15 inches inside the bark; Douglas fir to 10 inches inside the bark; and hemlock to 8 inches inside the bark.

No data were obtained concerning the yield of young or second-growth noble fir stands in the course of this study.

The logging of noble fir of the extreme size found presents an enormous amount of waste caused mainly by breakage. This is unavoidable in many instances, although a great deal of care is exercised by the tree fallers in placing the trees in falling. In addition to the breakage, considerable defect was found, especially in the larger sizes, in some instances necessitating culling of the butt log. Many of these large trees were found to be hollow on the stump, the rot extending up from 10 to 30 feet. Ring shakes were prevalent, scarcely any of larger trees being free of them. It is estimated that the deduction for defect and breakage would amount to from 20 per cent to 30 or 40 per cent of the total standing contents of the trees as given in Table VI.

TABLE VI.—*Comparative volumes of noble fir and associating species*

Diameter at breast height	Volume			Diameter at breast height	Volume		
	Noble fir [a]	Douglas fir [b]	Western hemlock [c]		Noble fir [a]	Douglas fir [b]	Western hemlock [c]
Inches	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*	*Inches*	*Bd. ft.*	*Bd. ft.*	*Bd. ft.*
30	2,220	1,250	1,830	46	6,200	4,250	4,890
32	2,650	1,450	2,115	48	6,840	4,800	5,255
34	3,100	1,690	2,450	50	7,500	5,350	5,595
36	3,560	1,950	2,840	52	8,180	5,875	5,920
38	4,040	2,275	3,255	54	8,900	6,450	6,240
40	4,540	2,700	3,680	56	9,630	7,050	6,560
42	5,070	3,120	4,095	58	10,400	7,650	6,880
44	5,630	3,650	4,500	60	11,170	8,300	7,200

[a] Noble fir utilized to a 15-inch top diameter inside the bark.
[b] Willamette Volume Table for Douglas fir. Trees utilized to a 10-inch top diameter inside the bark.
[c] Western Washington Volume Table for western hemlock. Utilized to an 8-inch top diameter inside the bark.

VOLUME

Noble fir, due to its long cylindrical bole and thin bark, has a greater volume for its diameter at breast height than any of its associating species, with the possible exception of western white pine. In Table VI a comparison of the board-foot contents of trees of equal diameter of noble fir, Douglas fir, and western hemlock shows plainly the greater volume of noble fir. The bark rarely exceeds 2 inches in thickness, averaging about 1½ inches on the stump for the larger trees. Douglas fir, on the other hand, has bark from 4 to 8 inches in thickness for corresponding sizes. The bark of hemlock is not any thicker than that of noble fir, but its bole is not nearly so cylindrical and the average length runs considerably less, which accounts for the smaller volume of hemlock for equal diameters.

THE GENETICS OF SEX IN HEMP[1]

By HUGH C. MCPHEE

Associate Animal Husbandman in Animal Genetics, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

In a recent paper (*7*)[2] the writer showed that the relative length of day and night exerts a profound influence on the development of both vegetative and floral parts of hemp. The results show that the length of time between planting and flowering in this species may vary from about 30 days to 90 days or more, depending on the relative length of day and night, other conditions being optimum for growth. The effect on the flowers was both quantitative and qualitative. When the period of daylight was long the plants were either pure carpellate or pure staminate, but when the period of daylight was short a number of intersex types appeared. Since the carpellate and the staminate flowers are normally produced on separate plants, such behavior is important because of its bearing on the mechanism of sex determination in this as well as in other dioecious species of plants.

There is no valid reason at present for doubting that environmental factors are largely responsible for the production of intersex types when hemp is grown in hothouses during the winter months. The results obtained by Schaffner (*10*, *11*, *12*) and by the writer (*7*) show quite clearly that individuals of either sex may produce flowers of the opposite sex when grown under such conditions. Sometimes such flowers are normal, but more often they are abnormal and sterile. The peculiarity of the case lies in the fact that some individual plants are affected much more than others grown under the same conditions and at the same time. In fact, some individuals do not produce flowers of the opposite sex under any conditions. The other extreme is reached in a case reported by the writer (*7*) in which a staminate-type plant matured several seeds. This is truly a reversal of the sexual function of the individual. This behavior, however, has led some botanists to suggest that sex in hemp is therefore nonmendelian in nature and can not be explained by a chromosomal mechanism. With such a suggestion the writer can not fully agree. The mere fact that the normal sexual expression of the individual is modified by certain factors does not prove that there is not a definite mechanism which is responsible for its development. It is merely the manifestation of the effect on the mechanism.

The point at issue, then, is whether or not the phenomenon of sex in hemp can be explained on a genetic basis. It would seem that the solution of this problem can be brought about only by a series of

[1] Received for publication March 20, 1925; issued January, 1926. The results reported in this paper were obtained at the Laboratory of Plant Genetics, Bussey Institution of Harvard University, and completed in the United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 942.

carefully planned breeding experiments in which the environmental factors are under the control of the operator. Published results of such experiments are conspicuous by their absence, and the presentation of data on this subject is the purpose of this paper.

PRELIMINARY OBSERVATIONS

Among plats of several varieties of hemp grown at the Bussey Institution of Harvard University during the summer and winter of 1921–22, the writer observed that in addition to the appearance of the usual intersex types in the hothouse during the winter there appeared a number of monoecious plants in the summer plat of the Simple Leaf variety. Almost without exception these individuals had the carpellate vegetative development and had been first classed as pure carpellate plants. The staminate buds were first observed when the seeds were about half mature. As a rule they were not restricted to any particular part of the plant, but were fairly well distributed among the seed-producing branches. Practically all staminate flowers thus produced were identical in appearance with those produced by pure staminate plants. Inasmuch as this plat of hemp was grown during the summer season, when the occurrence of "sex reversal" is rare, the production of functional flowers of both sexes on single individuals furnishes a starting point for breeding experiments to determine the extent to which such a phenomenon is hereditary. At first sight it may seem that such a case of "sex reversal" is different from that which occurs so commonly in hothouses during the winter. The two cases are fundamentally the same, however, and differ only in degree. The only difference lies in the fact that the abnormal flowers so common during the winter were absent from the monoecious Simple Leaf plants. The real question to be answered in either case is, what is the difference between the pollen produced by a carpellate type and that produced by a pure staminate type?

The sex ratios observed in the preliminary experiments (Table I) show a few peculiarities of minor importance. The most common condition seems to be about equal numbers of carpellate and staminate types. The minor fluctuations from this may be due to differential germination, death, or sampling of the seed. The sex ratio in the Simple Leaf variety, however, may be due to other causes not yet known. Since this variety arose as a mutation, it is suggested that its sexual state may be genetically different from that of the other varieties tested.

TABLE I.—*Sex ratios in different varieties of hemp*

Variety	Environment	Number	Sex ratio	Notes [a]
Chington	Field plat	472	105 : 100	No intersex types.
Kejo	do	136	106 : 100	Do.
Tochymington	do	341	130 : 100	1 intersex.
Simple Leaf	do	401	212 : 100	56 monoecious. [b]
Chington	Hothouse in summer	382	104 : 100	1 intersex.
Do	Hothouse in December–January	124	100 : 100	44 intersexes.
Do	do	103	93 : 100	17 intersexes.

[a] All plants which produced abnormal flowers are, for convenience, classes as intersexes.
[b] These plants were classed as carpellate in the sex ratio.

The intersex types (Table I) which occurred in the Chington variety were of various kinds, corresponding in general with those described by Schaffner (*9, 10, 11, 12*). In this connection it may be well to recall the fact already indicated that the growing of hemp in a hothouse does not, in itself, tend to bring about "sex reversal." Only one of the 382 Chington plants which were grown in a hothouse during the summer produced any flowers of the opposite sex. The proportion in the open plats was about 1 in 800. Both of these proportions are of relatively little significance, except that they demonstrate that "sex reversal" may occur during the long daylight period of summer. Although it is quite clear that relative length of day and night is an important factor in bringing about "sex reversal," it is not the only factor causing such changes.

BREEDING EXPERIMENTS

The general plan of the experiments was to make self and cross pollinations among the extreme intersex types in order to ascertain whether the tendency toward sex reversal was inherited. When the staminate buds were observed growing on what had been previously classed as pure carpellate Simple Leaf plants, the plats were visited several times daily, and when the buds were nearly ready to open each was removed, carefully brushed to remove foreign pollen and placed in a stoppered vial. These were kept in the laboratory until the anthers opened and liberated the pollen. This pollen was then transferred to stigmas of carpellate flowers which had been bagged for some time previously. Both pure carpellate and monoecious individuals were used. Three types of pollinations were made, namely, self-pollination, pollination of a pure carpellate with pollen from an intersex type, and cross-pollination of Chington stock plants as a check. From this series of pollinations a total of 326 seeds was obtained. When these seeds were planted in the greenhouse the results shown in Table II were obtained.

TABLE II.—*First generation of crosses between intersex types*

Variety	Number of seed planted	Carpellate	Staminate	Total
Simple Leaf intersex selfed	100	71	0	71
Simple Leaf carpellate × Simple Leaf intersex	64	56	0	56
Simple Leaf carpellate × Chington intersex	37	31	3	34
Chington carpellate × Chington staminate	148	46	42	88

It is at once obvious that there is a difference between the sex ratio of the check and that of each of the three other lots. The 88 plants in the check plat happened to give almost exactly a 1:1 ratio of the sexes, but of the other 161 plants, representing 3 series of pollinations, all except 3 were carpellate. The appearance of the 3 staminate individuals among the progeny of the Simple Leaf carpellate × Chington intersex cross is not in accord with the results of the other crosses.

The probable cause of this, however, is not difficult to find. The enormous amount of pollen which is produced by a single staminate hemp plant makes the elimination of contamination very difficult;

and although the work was conducted with great care there is a possibility nevertheless that a few stray pollen grains gained admission to the bagged branches of the Simple Leaf plants.

The 158 first-generation carpellate plants were grown in the hothouse during the winter months, and under these conditions several of them produced some staminate flowers; otherwise all were typically carpellate. The small amount of pollen which they produced was all used to make self-pollinations. As no staminate plants were used in the same house during the growth period, the chance of possible fertilization by pollen from a staminate plant was eliminated, It seems reasonable to assume, therefore, that any accidental pollination which might take place through carelessness would be a cross between two carpellate-type plants.

The number of staminate flowers available as sources of pollen was very small, and consequently the number of selfings which could be made was small. No cross-pollinations were attempted. The total number of seeds obtained was 193, many of which were small and shriveled. When these were planted the results shown in Table III were obtained.

TABLE III.—*Results from self-pollinating carpellate type intersex*

Cross	Seeds planted	Number dying young	Number of carpellate	Number of staminate
M-4 self	66	2	6	0
S-8 self	30	0	1	0
S-1 self	6	0	5	0
M-3 self	25	0	6	0
A-10 self	66	12	28	0
Total	193	14	46	0

The very poor germination of the seed is a most disappointing feature of these results. There is evidently a marked difference between the strains in this respect, but whether this is due to inherent characters or to other causes is a question which can not be answered from the data available. But of those which did germinate and grow to the flowering stage all were carpellate. Of course, these results do not prove that the progeny obtained by selfing a carpellate type intersex will all be of the carpellate type, but so far as they go they substantiate the results obtained in the previous generation.

Up to this point results obtained by selfing carpellate intersex types have been presented, and now it might be well to consider the possibilities of self-pollinating a staminate-type intersex. By this is meant a plant which is typically staminate until after the first few flowers have opened and then begins to produce also some flowers of the carpellate type. If this can be done it may yield some valuable information concerning the inherent differences between the carpellate and staminate types. For several reasons, such a plan holds forth relatively few chances of success. In the first place, the development of functional carpellate flowers on the staminate type intersex is much rarer than is the production of viable pollen by carpellate type intersexes; and, secondly, the inherent difference in the length of life of the two types makes it difficult to coax the staminate plant

to remain green long enough to properly mature seed. It will be recalled that the staminate type dies soon after the maturity of the pollen, while the carpellate type remains green and vigorous until the seed is mature.

By carefully regulating the relative length of day and night, and making drastic mutilations, the writer has succeeded in causing hemp which was at first typically staminate to produce functional carpellate flowers. The effects of the manipulations on the vegetative development were so great, however, as to make the plants very small and weak. It was, therefore, not advisable to bag them, and the seeds which they matured may have been the result of either cross or self-pollination. Only 11 seeds matured, and of these only 4 grew to the flowering stage. The sex distribution was 3 staminate and 1 carpellate Of course, these results are entirely too meager to warrant an extended discussion. The only significant fact brought out is that the staminate type carries the potentialities of the opposite sex type.

DISCUSSION

In the foregoing presentation of data, relatively little attention was given to the details of "sex reversal" which occurred during the winter, for the simple reason that they do not directly concern the immediate problem. The question to be answered concerns the mechanism of sex determination in hemp and not the effect of environmental or other factors upon this mechanism. When this much has been answered, then the time will be ripe for further inquiry into the details of each individual case of sexual modification.

The misinterpretation of the geneticist's conception of sex in dioecious plants has thrown some botanists into a chaos from which they have not yet emerged. It has been maintained that because a plant of one sex type develops some flowers of the opposite sex type, genetic factors are not concerned, and any question of homozygousness or heterozygousness is automatically ruled out. To substantiate such contentions abundant evidence is brought forth to prove that either sex type may produce flowers of the other type. With the facts shown by such data the writer agrees fully. The facts are fairly clear cut. There is a distinct vegetative dimorphism which is the same for both the sexual forms which remain pure and those which sooner or later show various sexual modifications. Under the ordinary cultural conditions found in the field these types occur in approximately a 1:1 ratio. The production of normal flowers of the opposite sex under such conditions, however, has been observed frequently. Among plants grown in the hothouse during the winter the sex ratio is approximately 1 : 1, but usually a large proportion of the plants develop flowers of the opposite sex. These flowers are usually abnormal. Such behavior is critical evidence that the normal development is being modified by environmental factors, but certainly should not be interpreted as meaning that certain genes are not responsible for the normal development of the character. There is abundant evidence from the well-known genetics of certain vegetative characters to show that the environment in which they develop may have such an effect as to prevent their expression at all. As Emerson (*5*) has pointed out, the mere fact that the expression of one character is influenced more by environment than is that of some

other characters does not signify that the genetics of the case is any less important.

The key to the situation is evidently to be found in the mechanism which controls the sex ratio under normal cultural conditions. The simple assumption that one sex is homozygous and the other heterozygous would explain the occurrence of the 1 : 1 sex ratio. A cross between such types is expected to produce equal numbers of each type. The data presented in Tables II and III indicate that the carpellate type is the homozygous one. Then, the carpellate form might be represented by the symbols XX and the staminate type by the symbols XY. The selfing of a carpellate type or crossing with another carpellate-type plant would be expected to produce only carpellate-type individuals. The selfing of a staminate-type individual would be expected to give rise to carpellate, staminate, and YY individuals in the ratio 1 : 2 : 1. The nature of the YY form, if such exists, is a question on which data are lacking at present. Otherwise, the data thus far obtained can be explained on such a provisional hypothesis. It is not to be assumed, however, that the condition is as simple as such a hypothesis might lead one to believe. The data available concerning the progeny of a selfed staminate-type plant consists of only 4 individuals, of which 3 were staminate and 1 was carpellate. This is entirely too small a number of individuals to be of any significance and can be of use only in suggesting that the staminate type is heterozygous for the sex factors. This is a point which must be cleared up by further investigation.

If the staminate type is hererogametic, it may be assumed that there exists a chromosome difference between the two kinds of gametes produced. Cytological investigations, however, have failed to show any such differences. The investigations of Strasburger (*14*) and of the writer (*8*) have shown that there is apparently nothing in the chromosome complex of hemp which can be identified as a sex chromosome. The 20 pairs of chromosomes are so nearly the same size and shape that it is not possible to follow them individually through the stages of cell division. But of course this evidence does not preclude the possibility that one pair of chromosomes is carrying the sex genes. There is no reason why the chromosome which carries the genes for sex should have a hook on one end or be larger than any of the others. This condition does exist in some animals and plants, but it does not follow that there is a correlation between the presence of sex genes and size or shape of the chromosomes which carry them.

During recent years a vigorous search for sex chromosomes in dioecious species of plants has been in progress. The results, positive in many cases, indicate that the male sex is heterogametic. Such a condition being in accord with the results obtained in breeding experiments with hemp, it seems desirable to mention the outstanding cases.

The presence of sex chromosomes in *Lychnis dioica* has been shown by Winge (*15*). The female plants contain 22+XX and the males 22+XY. This condition has been confirmed by Blackburn (*1*), who finds the diploid number to be 24, of which 2 are larger than the other 11 pairs. In the staminate plants, one of the pair differed from the other in respect to both length and shape. The interesting

results of breeding experiments reported by Correns (*2*, *3*), and the case of sex-linked inheritance of the XY type in this species reported by Shull (*13*), are in accord with the results of these cytological investigations. The presence of a visible chromosome difference in *Humulus lupulus* and in *H. japonicus* has also been shown by Winge (*15*). In both species the staminate type is heterogametic. So far as the writer is aware, there are no published results of breeding experiments to substantiate this finding.

Recently Kihara and Ono (*6*) have reported a peculiar chromosome condition in *Rumex acetosa*. The carpellate form contains 2 large M chromosomes and 12 autosomes. The staminate form contains 12 autosomes and 1 large "tripartite," which consists of a large M chromosome and two smaller elements. It is assumed that the large M is the X chromosome and the smaller elements the Y chromosome. Such a condition is in line with the results of Corren's (*4*) breeding experiments, from which he inferred that two kinds of pollen were being produced.

The evidence obtained from these sources shows that sex chromosomes exist in at least some of the dioecious species of plants. A fact of still greater importance is that both the genetic and cytological evidence points to the heterozygousness of the male form. It may seem, therefore, that a simple and general hypothesis should explain the mechanism of sex determination in such dioecious species. So little is known of the genetics of each species, however, that it seems to the writer that the accumulation of more genetic evidence should be awaited before a general hypothesis is formulated. For this reason the writer wishes to restrict to hemp his suggestions concerning sex in plants.

It has been repeatedly maintained during recent years that sex determination and segregation in dioecious species of plants has nothing to do with the segregation of the chromosomes, because sex is not determined until long after the reduction division and fertilization have taken place. Let us examine the evidence for such a contention in the case of hemp. It is not possible to distinguish between the carpellate and the staminate plants before the vegetative differences are developed or the flower buds appear. That is, during the early stages of growth all the plants appear to be the same. Is it to be assumed that the sex of such plants is not determined until just prior to the appearance of the flower buds? Is it to be assumed that in a plat of 10-day-old hemp the plants are neither staminate nor carpellate? The fact that it is impossible to distinguish the sexes one from the other at this stage of their life is not critical evidence that the sexual type has not been determined. If it could be shown, for instance, that in a plat of 1,000 plants all could be caused to develop into the carpellate type, there would be strong reason for believing that the sex of the individual is determined some time after the seed has germinated. But man has been unable to do this sort of thing, no matter what methods were used. Of course it has been shown repeatedly that as high as 90 per cent of plants in certain plats developed flowers of the opposite sex. Whether these flowers were the same genetically as those produced by the normal staminate or carpellate type is the really critical evidence which is lacking for these numerous cases. And until more of such evidence is available the

problem of sex determination in hemp as well as in other dioecious species of plants should be regarded with an open mind. The problem is so intricate that it probably will be fully solved only through the close cooperation between the geneticist and the physiologist.

SUMMARY

There is a distinct vegetative and sexual dimorphism in hemp. The vegetative differences are the same for those plants which remain sexually pure and those which later produce some flowers of the opposite sex.

The two sex types occur in approximately at a 1 : 1 ratio.

Under certain conditions both sex types produce flowers of the opposite sex. The most decisive factor in bringing this change about is the relative length of day and night.

When staminate and carpellate individuals are crossed, the progeny consists of staminate and carpellate in equal numbers. When the carpellate is selfed or pollinated with pollen from another carpellate type individual, the progeny are all carpellate. The seeds produced on a staminate plant give rise to both sex types.

It is maintained that sex determination in hemp can be explained on a genetic basis, and that the inheritance is of the XY type.

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EFFECTS OF FEEDING ANIMALS WITH TRICHINOUS MEAT CONTAINING NONVIABLE TRICHINAE [1]

By BENJAMIN SCHWARTZ,

Zoological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

In the extensive literature on trichinosis there appears to be no records of experiments dealing primarily with the effects produced by the ingestion of meat containing nonviable trichinae. Various workers have assumed, without adequate experimental evidence, that meat containing nonviable trichinae is innocuous in so far as the production of any of the symptoms that characterize trichinosis is concerned. Flury in 1913,[2] found that apart from the mechanical injuries caused by living trichinae in the host animal, the products formed as a result of their metabolic activities coincident with their growth and development in the host, as well as the products formed as a result of the degeneration of the muscles which they invade, are highly toxic to animals when injected parenterally. Flury, in fact, has explained the entire symptom complex of trichinosis, such as the gastric and intestinal disturbances, vomiting, local irritation, muscle stiffness, capillary hemorrhages, edema, blood changes, fever, and respiratory difficulties, as the result of (1) the toxic substances produced by the parasites, and (2) the degeneration products of the muscles which they invade. His careful studies, involving numerous experiments with various extracts of trichinous muscles, as well as the evidence furnished by various investigators regarding the toxicity of nematodes in general, naturally suggest the possibility that dead trichinae may also be injurious because of the liberation of possible toxic products as a result of their digestion, and that the ingestion of trichinous meat containing nonviable trichinae may give rise to at least some of the symptoms of trichinosis or to other unpleasant symptoms.

In order to determine the effects produced on animals by meat containing nonviable trichinae, several experiments were performed in which such meat was used. On account of their marked susceptibility to trichinosis, rats were chosen for the tests. Rats are likely to show severe symptoms not only after the parasites become established in the muscles but also during the intestinal stage, and usually die at this early stage of the disease even when they are fed very small quantities of heavily infested trichinous meat. Since rats frequently die of trichinosis before the larvae have begun to migrate, it may be assumed that death is due, in all probability, to a severe intoxication, coincident with the rapid growth and development of the parasites in the intestine, other probable contributory

[1] Received for publication June 19, 1925; issued January, 1926.

[2] FLURY, F. BEITRÄGE ZUR CHEMIE UND TOXIKOLOGIE DER TRICHINEN. Arch. Expt. Path. u. Pharmakol. 73: 164–213, illus. 1913.

factors being the effects of the severe irritation to the intestinal mucosa as a result of the rather deep penetration of the worms into the intestinal wall. If toxic substances are present in cooked and refrigerated trichinous meat in a state in which they are still capable of deleteriously affecting susceptible animals, the evidence of such toxicity should be demonstrable by feeding the meat to rats.

EXPERIMENTS

SERIES I

Meat from a trichinous rabbit, containing viable encapsuled worms, was gradually heated in water until the latter began to boil. Liberal portions of this meat were fed to each of six rats on August 16, 18, and 19, 1924. The animals devoured it readily. No symptoms developed as a result of the experiment, the animals continuing to eat the usual oat ration and exhibiting their normal activities for several months.

SERIES II

Trichinous pork that was very heavily infested was refrigerated for a period of 25 days at a temperature below 5° F., a procedure which is destructive to the vitality of trichinae. Liberal portions of this pork selected from parts of the carcass that were known to be heavily parasitized, namely, the diaphragm and the intercostal muscles, were fed to a series of 12 rats on December 3, 4, 6, 9, and 12, 1924. The rats showed no evidence of discomfort. They were kept alive on their usual ration for several months after the experimental feedings and continued throughout this period in apparent good health.

SERIES III

Liberal portions of cooked trichinous pork from a heavily infested hog were fed to 6 rats on January 10, 13, 19, 21, and 24, 1925. A sufficient quantity of meat was given each time to last from two to three days. All the meat given was consumed. No ill effects were observed, the animals continuing in good health for about two months after the experiments. Subsequently they were used in other experiments.

SERIES IV

The same animals that were used in Series III were fed trichinous pork refrigerated as described in connection with Series II. The rats were fed as follows: February 9, 1925, meat enough to last three days; March 6, 1925, enough to last two days; March 14, 1925, enough to last three days. The animals consumed all the meat that they were given and showed no ill effects. They were apparently in perfect health on May 25, 1925, on which date they were used in other experiments.

SERIES V

Three dogs and three cats were fed liberal portions of refrigerated trichinous pork, prepared in accordance with the method described in connection with the experiments in Series II. Sufficient meat was given each time to last from two to three days, no other feed being given during that period. The animals were fed on the follow-

ing dates: January 2, 4, 7, and 9, 1925. No ill effects were observed in these animals during the period of several weeks in which they were under observation.

DISCUSSION

On the basis of experiments described in this paper, in which relatively large quantities of meat containing nonviable trichinae were fed to animals with consistently negative results, it may be concluded that neither the dead trichinae themselves nor the muscles in which they were are encysted are injurious when ingested by animals susceptible to trichinosis, namely, rats, dogs, and cats. There is no reason to doubt the view that susceptible animals other than those used in these experiments, including human beings, would likewise be unaffected as a result of the ingestion of cooked trichinous meat or of trichinous meat refrigerated sufficiently to destroy the vitality of trichinae. If toxic substances are present in encapsuled trichinae and in the muscles in which they are lodged, these substances are either destroyed in the process of cooking and refrigeration or are broken down or neutralized in the course of digestion before they can exert deleterious effects on test animals.

The experiments described in this paper are in agreement with the commonly accepted view that after trichinous meat has been cooked sufficiently, refrigerated sufficiently, or treated by some other method known to destroy the vitality of trichinae, it is no longer capable of producing the symptoms characteristic of trichinosis. These experiments are useful in supplying an experimental verification of a view that has been commonly accepted on the basis of belief rather than on the basis of experimental proof.

SUMMARY

Rats, dogs, and cats were found to be tolerant of heavy doses of meat containing nonviable trichinae, exhibiting no ill effects as a result of repeated ingestion of such meat. These observations are in harmony with the generally accepted view that pork in which trichinae have been destroyed by cooking or by some other method known to devitalize trichinae will not produce any of the symptoms characteristic of trichinosis or other harmful effects.

LIMITATION OF STUDENT'S METHOD WHEN APPLIED TO FERTILIZER EXPERIMENTS[1]

By W. W. WEIR

Associate Soil Technologist, Bureau of Soils, United States Department of Agriculture

The advantages of Student's method for interpreting paired experiments are fully recognized by many agronomists largely because of the fact that this method brings within the range of statistical inquiry many crop and fertilizer tests the small-sample results of which furnish insufficient variability to enable one to judge of the significance or certainty of the results by the application of the usual probable-error formulas.

It has been pointed out by Love and Brunson [2] (*8*) that biometrical methods must be used with great care, that experimental data must be wisely and judiciously interpreted if the conclusions reached are to be of permanent value, and that Student's method is no exception to this rule. These same writers have also suggested that this method can be successfully applied only when the paired observations are made under similar conditions, or when the members of each pair are exactly comparable.

This present paper is presented with a view to illustrating, briefly, some of the limitations of Student's method when used in the interpretation of fertilizer results, and to calling attention to some precautions that should be taken in arriving at any mathematical expression of the significance or nonsignificance of one fertilizer treatment as compared with another.

Let the data in Table I be assumed for a pair of fertilizer tests on corn, obtained on two differently fertilized plots, A and B, belonging to the same series of tests:

TABLE I.—*Fertilizer tests on corn on differently fertilized plots*

Year	Plot A		Plot B	
	Yield per acre	Increase effected per acre	Yield per acre	Increase effected per acre
	Bushels	*Bushels*	*Bushels*	*Bushels*
1900	77.20	14	75.60	14
1901	53.50	12	49.70	11
1902	66.10	14	56.50	14
1903	65.80	10	63.80	11
1904	52.40	9	42.40	8
1905	57.30	13	47.70	14
Mean	62.05	12	55.95	12

[1] Received for publication Feb. 20, 1925; issued January, 1926.

[2] Reference is made by number (italic) to "Literature cited," p. 956.

When Student's method is applied to the paired *yields* a conclusion is reached showing the odds to be 195 to 1 that the fertilizer applied to plot A is more effective than the fertilizer applied to plot B. This can be true only when the natural soil productivity is a constant. However, if soil heterogeneity prevails to the extent that plot A represents a higher natural productivity than that represented by plot B, and this establishing a high degree of parallelism between the paired yields owing to the fact that yield A is always higher than B, in all probability the odds of 195 to 1 carry but little or no significance as regards the fertilizer applied to plot A.

Now let it be further assumed that the figures in the second column under A and B represent the actual increases effected in each case, these being entirely within the range of probability. In comparing the paired *gains*, either by a simple comparison of the data or by applying Student's method, it becomes plainly evident that no significance can be attached to the higher yields obtained on plot A, which are higher because of the difference in inherent productivity. It seems an absolute certainty that fertilizer A is not the more effective fertilizer, especially when cognizance is taken of the fact that the average gain effected by the one fertilizer is equal to the average gain effected by the other.

The foregoing illustration, though hypothetical, raises the question as to how Student's method should be applied to fertilizer results. Should the actual yields or the gains effected be paired?

It is a fact generally known that the soil constituting the plots of a series of tests in an experiment, though it be of the same type, usually varies more or less in its producing power on a given area, thus introducing the factor of soil heterogeneity in the interpretation of results. This condition, together with the fact that in but a few of the long-time fertility experiments already established are any of the fertilizer treatments repeated, makes it highly probable that the pairing of actual yields by Student's method may not establish certainty that one fertilizer treatment is more effective than another. This point is well illustrated by the published 18-year results obtained with wheat on plots Nos. 11 and 30 in the potato-wheat-clover rotation test at Wooster, Ohio, which follow (*1*, *3*, *4*, and Table II).

TABLE II.—*Student's method applied to wheat yields obtained in potato-wheat-clover rotation at Wooster, Ohio*

Year	Actual yields		Difference in yields, No. 11 over No. 30	Deviation from 5.68 (D)	D^2	Gains effected		Difference in gains, No. 11 over No. 30	Deviation from 1.88 (D)	D^2
	Plot No. 11[a]	Plot No. 30[b]				Plot No. 11	Plot No. 30			
	Bush.	*Bush.*	*Bush.*			*Bush.*	*Bush.*	*Bush.*		
1895	16.25	9.92	6.33	0.65	0.42	8.03	3.64	4.39	2.51	6.30
1896	15.20	5.66	9.54	3.86	14.90	8.14	−.42	8.56	6.68	44.62
1897	45.00	34.67	10.33	4.65	21.62	8.44	.72	7.72	5.84	34.11
1898	33.25	35.08	−1.83	7.51	56.40	9.36	10.39	−1.03	2.91	8.47
1899	39.00	30.50	8.50	2.82	7.95	9.75	6.67	3.08	1.20	1.44
1900	46.91	38.83	8.08	2.40	5.76	2.33	5.83	−3.50	5.38	28.94
1901	29.17	29.00	.17	5.51	30.36	3.56	6.59	−3.03	4.91	24.11
1902	55.00	41.52	13.48	7.80	60.84	16.47	10.99	5.48	3.60	12.96
1903	42.33	34.17	8.16	2.48	6.15	11.08	11.25	−.17	2.05	4.20
1904	35.96	31.54	4.42	1.26	1.59	14.89	9.79	5.10	3.22	10.37
1905	37.00	29.71	7.29	1.61	2.59	12.83	10.79	2.04	.16	.03
1906	52.33	43.50	8.83	3.15	9.92	6.02	5.92	.10	1.78	3.17
1907	42.75	37.42	5.33	.35	.12	8.03	2.70	5.33	3.45	11.90
1908	50.42	44.37	6.05	.37	.14	11.15	13.29	−2.14	4.02	16.16
1909	43.54	42.12	1.42	4.26	18.15	2.40	7.80	−5.40	7.28	53.00
1910	34.12	34.00	.12	5.56	30.91	11.44	8.30	3.14	1.26	1.59
1911	25.83	30.83	−5.00	10.68	114.06	12.24	13.61	−1.37	3.25	10.56
1912	18.04	6.92	11.12	5.44	29.59	6.52	.91	5.61	3.73	13.91
Mean	36.80	31.10	5.68		22.86 σ=4.80	9.04	7.15	1.88		15.88 σ=3.98

Z=1.18 (*5, p. 416*).
P=±0.9999+.
Odds=Over 10,000 to 1.

Z=0.471.
P=±0.9635.
Odds=26 to 1.

[a] Fertilizer treatment consists of 120 pounds of nitrate of soda, 50 pounds of dried blood, 160 pounds of acid phosphate, 100 pounds of muriate of potash, applied to wheat; and 80 pounds of nitrate of soda, 160 pounds of acid phosphate, and 100 pounds of muriate of potash, applied for potatoes.
[b] Fertilizer treatment consists of 8 tons of manure applied for potatoes.

Here it is shown that, because of the striking parallelism of the paired yields, the fertilizer treatment given to plot No. 11 is much superior to that given plot No. 30, this certainty being expressed by odds far exceeding 10,000 to 1. However, when the actual gains effected are paired, the parallelism is broken at seven different points, thus presenting in graphic form a more or less crisscross effect. Consequently, the odds are not sufficiently high to establish the fact that there is any important difference in the two treatments, odds 30 to 1 being accepted as the mathematical expression for establishing statistical significance.

In some cases when paired yields are subjected to Student's method for interpretation, odds indicate no degree of certainty that one fertilizer is better than another; but when the paired *gains* are subjected to the same test, odds are sufficiently high to enable one to come to quite a different conclusion. To illustrate this point, the published results obtained with corn for 20 years on plots Nos. 17 and 26 in the five-year rotation at Wooster have been selected, as follows (*1, 3, 4,* and Table III):

TABLE III.—*Student's method applied to corn yields in five-year rotation at Wooster, Ohio*

Year	Actual yields		Difference in yields No. 17 over No. 26	Deviation from .78 (D)	D^2	Gains effected		Difference in gains No. 17 over No. 26	Deviation from 4.31 (D)	D^2
	Plot No. 17[a]	Plot No. 26[b]				Plot No. 17	Plot No. 26			
	Bush.	*Bush.*	*Bush.*			*Bush.*	*Bush.*	*Bush.*		
1894	20.50	18.32	2.18	1.40	1.96	−0.62	1.80	−2.42	6.73	45.29
1895										
1896	56.89	59.43	−2.54	3.32	11.02	5.80	8.74	−2.94	7.25	52.56
1897	31.79	42.86	−11.07	11.85	140.42	13.11	9.48	3.63	.68	.46
1898	33.93	38.75	−4.82	5.60	31.36	8.06	7.24	.82	3.49	12.18
1899	40.50	34.50	6.00	5.22	27.25	18.81	10.36	8.45	4.14	17.14
1900	48.68	41.32	7.36	6.58	43.30	21.35	10.75	10.60	6.29	39.56
1901	60.64	63.14	−2.50	3.28	10.76	19.35	28.64	−9.29	13.60	184.96
1902	75.57	89.18	−13.61	14.39	207.07	23.25	22.92	.33	3.98	15.84
1903	22.32	23.43	−1.11	1.89	3.57	19.67	13.83	5.84	1.53	2.34
1904	40.04	34.46	5.58	4.80	23.04	22.95	19.40	3.55	.76	.57
1905	58.64	49.18	9.46	8.68	75.34	20.86	18.25	2.61	1.70	2.89
1906	63.32	64.54	−1.22	2.00	4.00	28.12	29.33	−1.21	5.52	30.47
1907	48.29	64.57	−16.28	17.06	291.04	12.98	17.56	−4.58	8.89	79.03
1908	44.96	43.64	1.32	.54	.29	20.16	10.51	9.65	5.34	28.52
1909	45.07	33.67	11.40	10.62	112.78	25.29	14.11	11.18	6.87	47.20
1910	17.07	12.03	5.04	4.26	18.15	13.09	3.19	9.90	5.59	31.25
1911	71.75	69.10	2.65	1.87	3.50	35.28	25.02	10.26	5.95	35.40
1912										
1913	42.50	26.25	16.25	15.47	239.32	29.87	8.58	21.29	16.98	288.32
Mean	45.70	44.90	+0.78		69.12 σ=8.31	18.74	14.43	+4.31		M=50.78 σ=7.12

Z=0.0939.
P=less than ±0.6575.
Odds=less than 2 to 1.

Z=0.61.
P=±0.9886.
Odds=87 to 1.

[a] Fertilizer treatment consists of 60 pounds of nitrate of soda, 25 pounds of dried blood, 160 pounds of acid phosphate, 100 pounds of muriate of potash, applied to wheat; and 80 pounds of nitrate of soda, 160 pounds of acid phosphate, and 80 pounds of muriate of potash, applied to corn and oats.

[b] Fertilizer treatment consists of 50 pounds of dried blood on wheat; 120 pounds, 240 pounds, and 240 pounds of nitrate of soda, applied on wheat, corn, and oats, respectively; bone meal equivalent to 160 pounds, 80 pounds, and 80 pounds of acid phosphate, applied to wheat, corn, and oats, respectively; and 100 pounds, 80 pounds, and 80 pounds of muriate of potash, applied to wheat, corn, and oats, respectively.

In this case a parallelistic analysis of the yields shows that the one fertilizer is practically as efficient as the other, but when Student's method is applied to the gains obtained, the odds are 87 to 1 that the fertilizer applied to plot No. 17 is more effective than that applied to plot No. 26.

It may be of interest to cite four other cases to illustrate how a conclusion reached by applying Student's method to paired yields may be reversed when the parallelisms of the corresponding gains are studied (Table IV).

TABLE IV.—*Student's method applied to paired yields and to the corresponding paired gains, in experiments at Wooster, Ohio*

Crop	Experiment and period of test	Plots compared	Fertilizer treatment	Interpretation of fertilizer results			
				Student's method applied to paired yields		Student's method applied to paired gains	
				Average yields	Statistical significance	Average gains	Statistical significance
				Bush.		*Bush.*	
Corn	Manure test, 3-year rotation, 1897 to 1913, inclusive.	2	Yard manure reenforced with rock phosphate.	62.60	Odds less than 2 to 1; acid phosphate as good as rock phosphate.	26.59	Odds greater than 10,000 to 1; acid phosphate is better than rock phosphate.
		5	Yard manure reenforced with acid phosphate.	61.80		31.50	
Wheat	Manure test, 3-year rotation, 1898 to 1913, inclusive.	2	do	24.50	Odds 18 to 1; no significance	12.48	Odds 3,332 to 1; acid phosphate is better than rock phosphate.
		5	do	25.40		14.85	
Do	Manure test, 3-year rotation, 1898 to 1913, inclusive.	6	Stall manure reenforced with acid phosphate.	25.89	Odds about 1 to 1; no significance	15.67	Odds 63 to 1; acid phosphate is better than rock phosphate.
		3	Stall manure reenforced with rock phosphate.	25.85		14.32	
Corn	Five-year rotation, 1895 to 1906, inclusive.	18	Manure, 8 tons on each corn and wheat.	39.94	Odds about 2 to 1; no significance	13.89	Odds 399 to 1; manure is the more efficient fertilizer.
		23	Complete chemical fertilizer on corn oats, wheat.	38.95		10.93	

In many experiments and probably in the majority of specific instances it does not matter which parallelism is used in the interpretation of results, since the application of Student's method to either will establish significance or nonsignificance in the same direction. In the foregoing examples it has been shown that the application of Student's method to paired gains may give odds that will not support the conclusion drawn when the method is applied to the corresponding pairs of yields, this being the way the method has been generally applied (*2; 6, p. 10; 7, p. 22; 8; 9; 10; 11, p. 6*). Moreover, when the interpretation of fertilizer results is based on the parallelism of yields it may even be shown that fertilizer treatment X, for example, is more effective than fertilizer treatment Y; but that when the interpretation of the results is based on the corresponding pairs of gains effected odds may indicate just about as high a degree of certainty that fertilizer treatment Y is the more effective. This may be illustrated by the published results obtained with wheat on plots Nos. 15 and 32 in the potato-wheat-clover rotation at Wooster for the period 1895 to 1912, inclusive, which follow (*1, 3, 4* and Table V):

TABLE V.—*Student's method applied to wheat yields in potato-wheat-clover rotation at Wooster, Ohio*

Year	Actual yields		Difference in yields No. 15 over No. 32	Deviation from 1.49 (D)	D^2	Gains effected		Difference in gains No. 32 over No. 15	Deviation from 2.16 (D)	D^2
	Plot No. 15[a]	Plot No. 32[b]				Plot No. 32	Plot No. 15			
	Bush.	*Bush.*	*Bush.*			*Bush.*	*Bush.*	*Bush.*		
1895		(c)								
1896		(c)								
1897	44.00	39.17	4.83	3.34	11.16	6.45	6.56	−0.11	2.27	5.15
1898	37.42	32.25	5.17	3.68	13.54	8.52	13.73	−5.21	7.37	54.32
1899	35.67	34.58	1.09	.40	.16	10.69	7.87	2.82	.66	.44
1900	48.00	44.91	3.09	1.60	2.56	13.91	5.59	8.32	6.16	37.95
1901	29.83	28.33	1.50	.01	.00	7.39	6.55	.84	1.32	1.74
1902	52.25	49.33	2.92	1.43	2.05	17.78	15.78	2.00	.16	.03
1903	38.67	38.17	.50	.99	.98	13.89	9.20	4.69	2.53	6.40
1904	27.50	29.21	−1.71	3.20	10.24	7.43	13.43	−6.00	8.16	66.59
1905	34.58	30.13	4.45	2.96	8.76	11.46	11.41	.05	2.11	4.45
1906	50.71	49.17	1.54	.05	.00	12.49	6.16	6.33	4.17	17.39
1907	35.00	40.83	−5.83	7.32	53.58	6.22	.44	5.78	3.62	13.10
1908	48.50	49.50	−1.00	2.49	6.20	17.99	12.06	5.93	3.77	14.21
1909	44.87	42.75	2.12	.63	.40	8.68	3.32	5.36	3.20	10.24
1910	33.54	34.12	−.58	2.07	4.28	9.10	11.72	−2.62	4.78	22.85
1911	39.25	36.33	2.92	1.43	2.04	17.94	14.36	3.58	1.42	2.02
1912	13.83	11.04	2.79	1.30	1.69	4.82	2.08	2.74	.58	.34
Mean	38.35	36.86	1.49		7.35 $\sigma=2.7$	10.92	8.77	2.16		16.08 $\sigma=4.01$
	$Z=0.55$. $P=\pm 0.9734$. Odds=37 to 1.					$Z=0.54$. $P=\pm 0.9715$. Odds=34 to 1.				

[a] Fertilizer treatment consists of 32 pounds of nitrate of soda, 480 pounds of acid phosphate, and 300 pounds of muriate of potash per acre applied for potatoes only.
[b] Fertilizer treatment consists of 16 tons of barnyard manure applied for wheat.
[c] No yields are reported for plot No. 32 for 1895 and 1896.

When Student's method of interpretation is applied to the actual yields obtained on plots Nos. 15 and 32 certainty is established in terms of odds of 37 to 1 that the complete chemical fertilizer applied for potatoes, on plot No. 15, is more effective in increasing the yields of wheat (the crop following potatoes) than 16 tons of manure

applied directly for wheat, on plot No. 32; and that when the parallelism of the gains is subjected to the same method of interpretation exactly the opposite conclusions are reached, namely, the odds are 34 to 1 that it is the manure and not the chemical-fertilizer treatment which is the more effective.

It is not the aim here to discredit Student's method. Its advantages are fully recognized. Nor is it the intention of the writer to raise at this time any question as to the certainty of significant differences in fertilizer effects, especially in those cases where, largely because of soil heterogeneity, the interpretation, when based on the parallelism of gains, leads to conclusions opposite to those arrived at when the interpretations are based on paired yields. (This latter point presents a distinct problem, since it is well known that the response made by a soil type to a given fertilizer treatment will vary more or less on local areas or spots, depending in a large measure upon the kind of fertilizer and the degree of difference in the natural producing power of the soil.) The writer wishes simply to call attention to the fact that this method of interpreting fertilizer results has its limitations.

The facts are that natural soil productivity is not a constant on all the plots of a fertility experiment and that the application of Student's method to paired yields may not lead to correct interpretations of fertilizer results. When Student's method is applied to yields soil homogeneity is assumed, but when the method is applied to paired gains soil heterogeneity enters into consideration. Thus it seems more correct statistically and more logical (in order to meet the conditions under which many fertilizer experiments are conducted) to apply Student's method, wherever possible, to parallel gains as a better test of the certainty of any significant difference in fertilizer effects.

A study of the principles on which Student's method is based and of the statistical formulas on which its proof rests can hardly lead one to restrict its application to paired yields. Furthermore, in experiments in which the effects of different fertilizer treatments are compared, the interest does not center so much on the yields as on the gains effected, particularly when such gains can be expressed as "deviation from normal," or deviations from control-plot treatment, assuming progressive differences between control plots. Thus from both the practical and economic points of view it is the difference between gains effected, or second differences, which finally determines which of two fertilizer treatments is the more effective and also perhaps the more profitable.

It has long been the practice to measure the comparative values of different fertilizers in terms of margin of profit over the cost of the fertilizers as determined by the market value of the increases obtained. This is highly desirable from the economic or profit point of view. However, there remains to be given alongside these profits, which serve to measure the comparative economic values of any two fertilizers, some mathematical expression of the statistical significance of the effects of one fertilizer treatment when compared with the other. This can be done consistently, and probably with less error as affecting conclusions, when the interpretation of results is based on the parallelism of gains rather than of actual yields.

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CROWN-GALL STUDIES OF RESISTANT STOCKS FOR PRUNUS[1]

By CLAYTON O. SMITH

Research Associate in Plant Pathology, Citrus Experiment Station, College of Agriculture, University of California

INTRODUCTION

Of all nursery troubles in California on the stocks used for the stone fruits, the disease commonly known as crown gall is probably the most prevalent. Observations in the nursery often reveal a high percentage of infection with crown gall, especially in sandy soils, on peach and almond roots, and to a less extent on myrobalan plum and apricot.

Investigation (*3*)[2] has shown that the nursery stocks in general use for the stone fruits in California are susceptible to artificial inoculation with the crown-gall organism, *Pseudomonas tumefaciens* Sm. and T. Cannery pits of peach and apricot, rather than pits of the more primitive species, are usually planted for such nursery stock. Inoculations of the peach (*Amygdalus persica*) produce about 80 per cent infection, and the almond (*Amygdalus communis*) is probably as susceptible. Inoculations of the myrobalan plum (*Prunus cerasifera*) result in 75 to 95 per cent of galls. The apricot (*P. armeniaca*) is somewhat more resistant, infections resulting from about 74 per cent of the inoculations.

The general characteristics of the crown-gall disease caused by *Pseudomonas tumefaciens* have been carefully studied (*6*), but a satisfactory and practical control is yet to be found. The most promising line of attack is in the use of more resistant stocks, species, or varieties of Prunus or Amygdalus which in experiments or practice have shown themselves substantially free from infection, and which are also suitable for growth.

The genus Prunus is rich in species and varieties which might be employed for stock purposes. Little is known concerning their adaptability as roots for the stone fruits or their resistance to crown gall; these questions are under investigation at the present time. This paper gives a preliminary report on the inoculation of a number of species and varieties of Prunus and Amygdalus.

SOURCE OF MATERIAL

PRUNUS FORMS

The species listed in Tables I and II have been obtained from supposedly reliable sources. Scions and pits of many of the eastern varieites were obtained from the Arnold Arboretum. The United States Department of Agriculture has sent us material from its

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[2] Reference is made by number (italic) to "Literature cited," p. 971.

foreign and domestic introductions. Several varieties were obtained from reliable nurserymen. The wild varieties of the Pacific States were obtained through the cooperation of forestry supervisors or through personal collection of pits from natural plantings. W. F. Wight furnished pits of some of the eastern varieties.

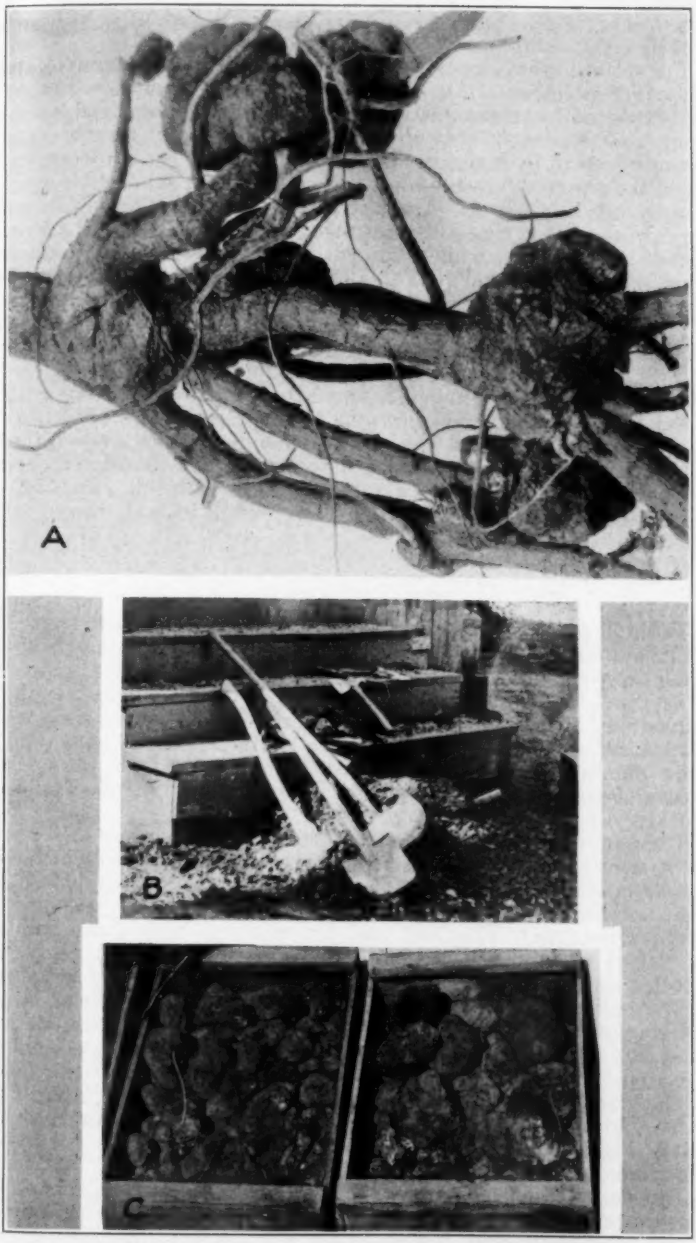
In Tables I, II, and VIII the following letters are used to indicate the difference sources from which the plant material used in this study was obtained:

AA—Arnold Arboretum, Jamaica Plains, Mass. (Seeds and scions.)
ESC—Author's collection, Citrus Experiment Station, University of California. (Seeds and scions.)
ESK—Kansas Agricultural Experiment Station. (Seed from Albert Dickens.)
ESO—Oregon Agricultural Experiment Station. (Scions from H. P. Barss.)
EST—Texas Agricultural Experiment Station. (Seed and scions from H. Ness.)
NB—Luther Burbank Nursery, Santa Rosa, Calif. (Seedlings.)
NC—Leonard Coats Nursery, Morgan Hill, Calif. (Budded trees.)
NF—Fancher Creek Nursery (Geo. C. Roeding, manager), Fresno, Calif. (Budded trees.)
NLC—L. R. Cody Nursery, Saratoga, Calif. (Scions.)
NP—Theodore Payne Nursery, Los Angeles, Calif. (Seeds.)
NS—Shenandoah Nursery, Shenandoah, Iowa. (Seedlings and budded trees.)
NT—Tribble Brothers Nursery, Elk Grove, Calif. (Root cuttings.)
UCP—C. A. Parpus through Department of Botany, University of California. (Seed.)
USDA—United States Department of Agriculture, Office of Seed and Plant Introduction. (Seedlings and budded trees.)
USDAF—United States Department of Agriculture, Forestry Service. (Seeds.)
USDAH—United States Department of Agriculture, San Antonio Experimental Farms, Texas. (Buds from H. S. Hastings.)
USDAM—United States Department of Agriculture, Bureau of Plant Industry, Silas C. Mason. (Seed.)
USDAW—United States Department of Agriculture, Bureau of Plant Industry, W. F. Wight. (Seeds.)

The different species were verified by a careful comparison with scientific descriptions, and in doubtful cases were further authenticated by submitting material to a botanist who had specialized in the genus Prunus. The horticultural varieties were studied critically but in many cases could not be positively identified because of their failure to fruit. This is especially true of the varieties of *P. domestica*. (See Table II.) Italian Prune, German Prune, Reine Claude (Green Gage), President, and Bartlett fruited and were apparently correctly named. The other varieties in Table II were not authenticated, but the names were used as labeled by nursery.

THE CROWN-GALL ORGANISM

The crown-gall organism used in 1913 and 1914 in the inoculations on Prunus was isolated from the almond. The organism was reisolated in 1914 from *Schinus molle*, and in 1916 and 1918 from two species of Prunus. The cultures used were always in a virulent condition, as indicated by the control inoculations, which resulted in 80 to 100 per cent infection. They were never more than two years from isolation. In 1921 a new culture, the last used, was obtained from a natural gall found on *Prunus besseyi*. In all, five different cultures have been used, three of which were from artificial galls produced by the original culture or by cultures reisolated from them.



A.—Galls which developed in two years on a small seedling of *Amygdalus davidiana*. Inoculation by mixing fragments of crown galls with soil at time of planting
B.—A pile of crown-gall fragments as they were prepared for use in inoculating soil in these experiments
C.—Boxes showing nature of gall material before being broken up for use in the experiments

EXPERIMENTAL METHODS

For several years inoculation tests have been made each week from May to September (*4*), using pure cultures of *Pseudomonas tumefaciens* isolated from almond, peach, or plum. The artificial inoculations were made in series of 10 or a multiple of 10 on the current season's growth by punctures with a steel needle. No protection was given the puncture inoculations. In each series a susceptible species was included to test the virulence of the culture. The results were very consistent, in that a resistant species usually showed the formation of but few galls, while a susceptible one showed a large number. This is well illustrated in Table VI, which presents the results of several series of inoculations.

In addition to the method outlined above, pits of such species (Table VII) as could be obtained were planted in soil with fragments of galls taken from diseased orchard trees, usually peach roots (pl. 1). These galls were broken up into small fragments by pounding with a heavy tool. The pits were sown the proper distance apart in a furrow 3 or 4 inches deep, and the gall fragments scattered upon them. The pits in the upper part of each row were not inoculated, being left as a control. The furrow was then filled with soil. In another experiment one-year-old trees were planted in soil, mixed with broken pieces of crown gall.

RESISTANCE IN INOCULATION TESTS

Table I presents a summary of the results obtained from the artificial inoculations on species of Prunus. These results agree with those previously published (*5*). In some cases the number of inoculations was too small to make the results entirely conclusive; these results may be considered as only suggestive of the actual resistance. A resistant species usually shows the characteristic at once, and a large number of inoculations, while making the test more conclusive, generally does not change very materially the actual percentage of galls produced.

The name Prunus is used in this paper in its inclusive sense, and the species tested are very diverse. Three species, *Prunus ilicifolia*, *P. integrifolia*, and *P. caroliniana*, are evergreens, and are placed by some authorities in Laurocerasus. It will be noted that these showed strong resistance. They can not, however, be successfully budded or grafted with any of the cultivated varieties of the stone fruits. Another group of native subdesert species, *P. andersoni*, *P. fasciculata*, *P. fremonti* (*P. eriogyna*), *P. minutifolia*, and *P. microphylla*, while differing among themselves, have certain common characteristics. They are about equally resistant, yielding about 50 per cent of infections. With the exception of the last two species, they grow in the Pacific and Rocky Mountain States. They are interesting forms, but are probably too small to be valuable as standard stocks. These, together with *P. subcordata*, the Sierra plum, are worthy of experimental testing as dwarf stocks.

The varieties of the species *Amygdalus persica* and *A. communis* thus far tested have shown no marked resistance. The bitter almond is the most promising, but in actual nursery practice this variety often is very susceptible. Other species of Amygdalus, as *A. mira*

and *A. tangutica*, appear to be more resistant, but so far only a single introduction has been tested by inoculation, and the inoculations of a larger number of seedlings may change their status in this respect.

TABLE I.—*Summary of artificial inoculations on species of Prunus and Amygdalus by cultures of Pseudomonas tumefaciens*

Species	Common name	Source [a]	Number of inoculations	Number of galls	Per cent of infection
A. communis L.	Bitter almond	NF	190	56	29.4
A. davidiana Zabel	Davidiana peach (S. P. I. 36664).	USDA	250	130	52.0
A. mira (Koehne)	S. P. I. 34601	USDA	650	120	18.5
A. persica L.	Peach	ESC	211	187	88.6
A. persica	Indian cling peach seedlings.	EST	610	396	64.9
A. persica	Peach (S. P. I. 40001)	USDA	250	79	31.6
A. persica potanini Ricker	Peach (S. P. I. 40009)	USDA	200	50	25.0
A. tangutica Korsh	Chinese wild almond (S. P. I. 40010).	USDA	430	25	5.8
P. alleghaniensis Porter	Allegheny plum	AA	250	12	4.8
P. andersoni A. Gray	Desert peach	USDAF	70	30	42.9
P. angustifolia Marsh	Chickasaw plum	ESC	140	37	26.4
P. angustifolia watsoni Waugh	Sand plum	ESK	180	69	38.3
P. americana Marsh	American plum	AA	180	129	71.6
P. armeniaca L.	Apricot (S. P. I. 32834)	USDA	60	5	8.3
P. armeniaca	Blenheim apricot	NF	80	76	95.0
P. armeniaca	Royal apricot	NF	170	140	82.3
P. armeniaca	Royal apricot seedlings	ESC	50	39	78.0
P. armeniaca	Tilton apricot	NF	70	52	74.3
P. armeniaca	Apricot (S. P. I. 17154)	USDA	140	60	42.8
P. armeniaca mandshurica Maxim.		AA	170	39	22.9
P. besseyi Bailey	Bessey cherry	NS	280	29	10.4
P. besseyi Hybrid	Sapa plum	NS	90	54	60.0
P. besseyi Hybrid	Compass plum	NS	50	12	24.0
P. caroliniana Ait	Cherry-laurel	NP	150	0	0.0
P. cerasifera Ehrh	Myrobalan plum	NF, AA	950	725	76.3
P. cerasifera divaricata Bailey		AA	100	94	94.0
P. cerasifera pissardi Koehne	Purple-leaf plum (seedling).	ESC	50	35	70.0
P. cerasifera Hybrid	Marianna	NS	90	59	65.6
P. dasycarpa Ehrh	Purple apricot	AA	210	94	44.8
P. domestica L.	See Table II				
P. emarginata villosa Sudworth		ESO	40	39	97.5
P. fasciculata A. Gray	Desert almond	ESC	20	4	20.0
P. fremonti S. Wats	Desert apricot	USDAM	90	48	53.3
P. hortulana Bailey	Hortulana plum	AA	130	108	83.1
P. hortulana	Golden Beauty plum	AA	190	51	26.8
P. ilicifolia Walp	Hollyleaf cherry	NF	150	0	0.0
P. insititia L.	Shropshire plum	NF	230	35	15.2
P. insititia	St. Julian plum	NS	110	11	10.0
P. lyoni Sarg	Catalina cherry	NP	80	3	3.7
P. maritima Marsh	Beach plum	AA	140	48	34.3
P. mexicana S. Wats	Big-tree plum	USDAW	280	167	59.6
P. microphylla Hemsl	Mexican almond	UCP	60	29	48.3
P. minutiflora Engelm	Texas almond	USDAH	60	30	50.0
P. munsoniana Wight and Hedr.	Wildgoose plum	AA	160	118	73.7
P. mume Sieb. and Zucc.	Japanese apricot	USDA	4,950	307	6.0
P. nigra Ait.	Canada plum	AA	80	76	95.0
P. orthosepala Koehne		AA	90	80	88.8
P. pumila L.	Sand cherry	USDAW-AA.	450	1	0.2
P. serotina Ehrh	Black cherry	NB	150	24	16.0
P. simoni Carr	Apricot plum	NF	130	130	100.0
P. spinosa L.	Blackthorn plum	NF	80	57	71.2
P. subcordata Benth	Sierra plum	USDAF	15	3	20.0
P. tomentosa Thunb	Nankin cherry	AA	25	22	88.0
P. umbellata Ell	Black sloe (S. P. I. 38974)	USDA	150	1	0.6
P. umbellata	Seedlings (S. P. I. 38974)	ESC	690	173	25.0
P. umbellata injuncunda Sarg		AA	25	22	88.0

[a] The explanation of the letters used in Tables I, II, and VIII may be found under "Source of Material."

Prunus armeniaca has not thus far shown any marked resistance in any of the horticultural varieties tested. *P. armeniaca mandshurica*, considered by some a distinct species, is somewhat resistant.

The introduction *P. armeniaca* (S. P. I. 32834) also seems promising, but has not been sufficiently tested.

The varieties of the European plum (*Prunus domestica*) are placed together in Table II. This species contains a great diversity of varieties, some very resistant and others rather susceptible. Six of the seventeen tested show 15 per cent or less of galls developing from artificial inoculations. Is this what may be expected when a number of different varieties of a species are tested by artificial inoculations? *P. insititia*, the damson group, is in many respects very similar to *P. domestica*. It shows a very satisfactory resistance.

TABLE II.—*Artificial puncture inoculations on varieties of Prunus domestica, arranged in order of resistance*

Variety	Source [a]	Number of inoculations	Number of galls	Per cent of galls
Italian Prune	NF	340	13	3.8
Golden Drop	NF	160	14	8.7
German Prune	NF	350	31	8.8
Washington	NC	60	6	10.0
President	NC	140	19	13.5
Giant	NC	60	9	15.0
P. D. stock [b]	NT	420	77	18.3
Columbia	NC	80	20	25.0
Black Damask	NLC	20	6	30.0
Reine Claude (Green Gage)	NF	160	62	32.5
Sergeant	NC	130	49	37.7
Red Magnum Bonum (Red Egg)	NF	80	26	38.7
Jefferson	NC	110	49	44.5
St. Martin	NC	130	81	62.3
Grand Duke	NC	60	42	70.0
Bartlett	NC	60	51	85.0
Sugar	NF	70	67	95.7

[a] The explanation of the letters used in Tables I, II, and VIII may be found under "Source of material."

[b] Stock of *Prunus domestica*, used to a limited extent in California. This material was obtained as suckers from orchard trees budded or grafted on this root. The stock can be propagated rather easily by root cuttings.

SOME RESISTANT SPECIES

The species showing marked resistance are listed in Table III, and, with the exception of *Prunus ilicifolia*, *P. caroliniana*, and *P. serotina*, merit further field tests as stocks for the stone fruits. Some of them have been used in a limited way, while others probably have never been given experimental trial as stocks.

TABLE III.—*Species of Prunus and Amygdalus showing less than 16 per cent infection by artificial inoculations with pure cultures of Pseudomonas tumefaciens*

Species	Common name	Number of inoculations	Number of galls	Per cent infection
P. illicifolia	Holly-leaf cherry	150	0	0.0
P. caroliniana	Cherry-laurel	150	0	0.0
P. pumila	Sand cherry	450	1	0.2
P. umbellata	Black sloe	150	1	0.6
P. domestica [a]	Italian prune	340	13	3.8
P. alleghaniensis	Allegheny plum	250	12	4.8
A. tangutica	Chinese wild almond	430	25	5.8
P. mume	Japanese apricot	4,950	307	6.0
P. domestica	Golden Drop plum	160	14	8.7
P. domestica	German prune	350	31	8.8
P. besseyi	Sand cherry	280	29	10.4
P. insititia	St. Julian plum	110	11	10.0
P. serotina	Black cherry	150	21	14.0
P. insititia	Shropshire plum	230	35	15.2

[a] Only one of the six varities listed in Table II as showing 16 per cent or less infection is given here.

Four different seedlings of the sand cherry, *P. pumila*, have been used in the inoculation tests, and it seems to be somewhat more resistant than the variety of *P. besseyi*, known as the Rocky Mountain Dwarf. This stock was purchased from an eastern nursery, and it is not known whether the inoculations were made on seedlings or on stock grown from cuttings from a single source. *P. pumila* grows readily from cuttings planted in the spring, and the same is probably also true of *P. besseyi*. The black sloe (*P. umbellata*) is rather promising. It produces a good-sized tree and does not sucker badly. *Amygdalus tangutica* may be valuable as a dwarf peach and almond stock, though it is said to sucker freely, which is an objection. *P. mume* is the most promising species, and will be considered later. *Amygdalus mira* (the smooth-pit peach) and *A. persica potanini* are promising. They seem to be vigorous growers and should be further tested.

The results are given in Tables I to III can be summarized by arranging the species of Amygdalus and Prunus in the following groups:

(1) Amygdalus: *mira, tangutica,persica, communis;* 3 to 88 per cent.
(2) Evergreen species (Laurocerasus): *caroliniana, ilicifolia, lyoni;* 0 to 3 per cent of infection.
(3) Sand plums: *pumila, besseyi;* 0.2 to 10 per cent.
(4) *Domestica:* 17 varieties tested (Table II); 3 to 95 per cent.
(5) Damson (*insititia*): St. Julian, Shropshire; 10 to 87 per cent.
(6) Subdesert species: *andersoni fasciculata, fremonti, microphylla, minutifolia;* 20 to 50 per cent.
(7) Sloelike plums: *alleghaniensis, spinosa, umbellata* (S. P. I. 38974 and seedlings of *umbellata* (S. P. I. 38974); 0.6 to 88 per cent.
(8) Chickasaw plum: *angustifolia, angustifolia watsoni;* 24 to 37 per cent.
(9) Myrobalan plum (*cerasifera*): seedlings, var. *divaricata*, and seedlings of var. *pissardi;* 75 to 90 per cent.
(10) Apricot (*armeniaca*): Royal, Royal seedlings, Blenheim, Tilton, etc., and var. *mandshurica;* 8.3 to 95 per cent.
(11) Japanese apricot (*mume*): 46 seedlings (Table V); 1.5 to 13 per cent.

The Black Sloe (Prunus umbellata)

This species is one of the most promising of the native forms of Prunus for use as a rootstock for the stone fruits. It has been used for rootstocks in Florida. In California, under the climatic conditions of Riverside, it makes, the first season, a stocky growth of 3 to 4.5 feet in height. Tests are under way to determine its adaptability for the different varieties of the stone fruits. The results of the artificial inoculations as listed in Table I showed a single tree of *P. umbellata* (S. P. I. 38974) as less susceptible than *P. umbellata injucunda* (*P. mitis* Beadle), but further inoculation tests of the former on more rapidly growing wood are necessary before its crown-gall resistance can be considered as established.

In Table IV are given the tabulated results of inoculation of seedlings of *Prunus umbellata* (S. P. I. 38974). Control inoculations were made on seedlings of Indian Cling peach. Eighty puncture inoculations gave 53 galls or 66 per cent infection, which result agrees very closely with 64.9 per cent given for this form of *P. persica* in Table I.

TABLE IV.—*Results of artificial inoculations in 1924, on seedlings of Prunus umbellata (S. P. I. 38974)*

Number of seedlings	Age (years)	Number of inoculations	Number of galls	Per cent of infection
1	3	90	0	0.0
1	3	90	2	2.2
1	3	110	3	2.7
1	2	60	5	8.3
1	2	60	5	8.3
1	2	60	5	8.3
1	3	70	9	12.8
1	3	80	23	28.7
1	3	80	28	35.0
1	3	110	44	40.0
1	3	100	49	49.0
11	---------	910	173	[a] 19.0

[a] Average.

The seedlings whose inoculations are listed in Table IV were all growing vigorously, and were in favorable condition to give a high per cent of crown-gall infection. The seedlings showed considerable variation in their susceptibility, but in most cases they apparently were more susceptible than the parent tree. More than half of them (7) showed less than 16 per cent infection, and could well be listed with the resistant forms as given in Table III. The results suggest, at least, that with proper selection a resistant form of this species could be developed. An asexual method of propagation would of necessity have to be employed. From a limited test, the root-cutting method seems promising.

THE JAPANESE APRICOT (PRUNUS MUME)

The species most thoroughly tested is the Japanese apricot, *Prunus mume*. The results of the tests are summarized in Table V. Two of the introductions, S. P. I. 28685 and 43558, were budded on other stock. The other three introductions were seedlings grown from pits imported through the Bureau of Plant Industry of the United States Department of Agriculture. The pits from S. P. I. 46694 and 47950 were grown on the California Citrus Experiment Station grounds. S. P. I. 45523 consisted of 25 small trees obtained from the United States Department of Agriculture. It will be observed (Table V) that there is considerable variation in the percentage of galls that developed. S. P. I. 46694 is exceedingly low in this ratio, while S. P. I. 45523 is relatively high. The other three are practically alike. This variation in susceptibility can also be noted in the individual trees of introduction S. P. I. 45523. The trees of the other introductions are all practically alike in susceptibility, being very resistant.

The galls that develop on the resistant trees of *Prunus mume* are small in size, sometimes mere pointlike growths that have formed on the healing tissue at the margin of the wound. These are usually less than one-sixteenth inch in height, with about the same diameter at the base. As to the shape, they are pointed rather than subglobose. It is very probable that most of these smaller pointlike galls will not further increase in size. Resistance, while suggested

by the small size and characteristic shape of the galls, is demonstrated by the small number that develop. These characteristics of gall development have been observed (*3*) in other resistant species, especially in the case of *P. domestica* (Italian prune and German prune) (pl. 3, B to E).

TABLE V.—*Summary, by years of inoculations, on different introductions of Prunus mume*

Year	S. P. I. 28685 [a] (1)	S. P. I. 43558 [a] (1)	S. P. I. 45523 [a] (21)	S. P. I. 46694 [a] (12)	S. P. I. 47950 [a] (11)
1916	[b] 30 0				
1917	120 10				
1918	120 16				
1919	60 1	[b] 40 8	[b] 70 13		
1920	20 0	20 0		[b] 150 0	[b] 600 34
1921	70 5	70 8	420 54	620 20	260 20
1922	80 8	50 0	840 122	810 4	530 2
Total	500 40	180 16	1,330 189	1,580 24	1,390 56
Per cent of galls	8.0	8.9	14.2	1.5	4.0

[a] The catalogue number assigned by the Office of Seed and Plant Introduction, Bureau of Plant Industry, United States Department of Agriculture. The number in parentheses indicates the number of different seedlings inoculated.

[b] The first number indicates the number of inoculations, the second the number of galls that developed.

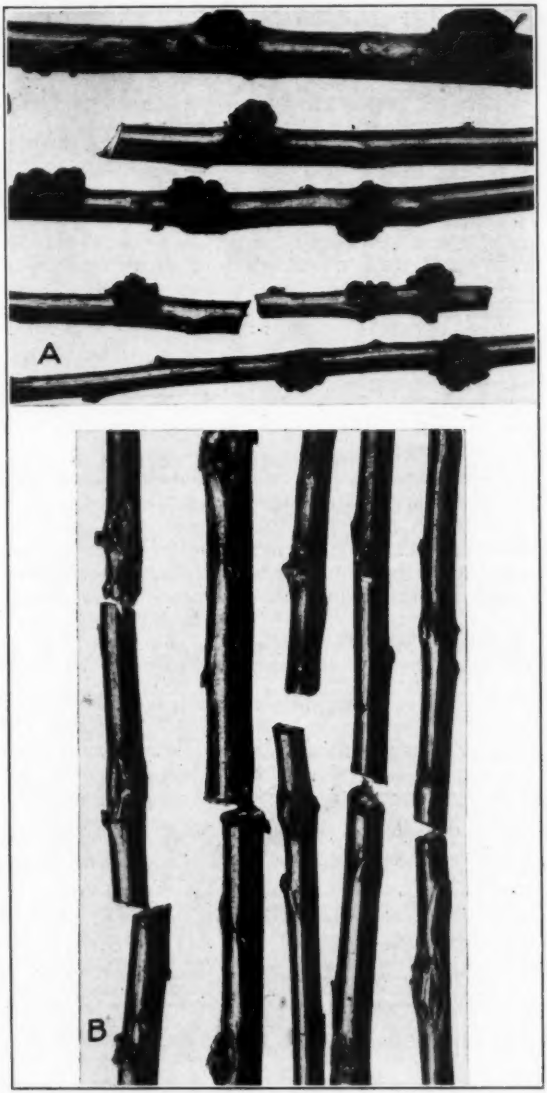
On the more susceptible introductions of *Prunus mume* the galls are much larger and subglobose in shape. They are similar to typical crown galls that develop on other hosts from artificial inoculations.

TABLE VI.—*Number of galls which developed in each series of 10 punctures in inoculated species, resistant and susceptible, of Prunus and Amygdalus*

Species	Common name	Number of galls developing from each 10 punctures [a]
P. domestica	Italian prune	0, 1, 0, 0, 0, 0, 0, 4, 0, 2, 0, 0, 2, 0, 0, 3, 0, 0, 0, 0, 1, 0
P. domestica	German prune	0, 0, 0, 0, 6, 0, 0, 6, 0, 2, 0, 0, 0, 0, 0, 2, 0, 0, 0, 0.
P. besseyi	Sand cherry (Rocky Mountain Dwarf).	1, 0, 2, 2, 0, 0, 4, 0, 0, 0, 0, 0, 0.
P. mume	S. P. I. 46694 (Japanese apricot.)	0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 6, 0, 2, 2, 0, 2, 0, 0, 0, 0, 1, 0, 0, 1.
P. mume	S. P. I. 47950 (Japanese apricot.)	3, 0, 3, 8, 7, 6, 0, 5, 3, 5, 1, 0, 0, 1, 4, 4, 1, 0, 0, 2, 1, 0, 0, 1, 0, 1, 0, 0.
P. angustifolia watsoni		10, 0, 0, 5, 0, 0, 6, 6, 7, 4, 3, 4.
P. alleghaniensis	Allegheny plum	0, 0, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 3, 3, 0, 5.
A. tangutica	Chinese wild almond	0, 3, 1, 0, 1, 0, 0, 0, 0, 1, 1, 4, 0, 1, 0, 6, 6, 1, 0, 0, 0, 0, 1, 0, 0.
P. cerasifera	Myrobalan plum	10, 8, 9, 8, 5, 8, 0, 4, 2, 4, 10, 2.
P. cerasifera divaricata		10, 10, 9, 7, 10, 8, 10, 10, 10, 10.
A. persica	Elberta peach	9, 10, 10, 10, 9, 0, 10, 10, 7, 8, 0, 10, 10, 10, 10.
A. communis	Bitter almond	8, 10, 0, 2, 0, 0, 0, 0, 0, 0, 8, 7, 0, 4, 0.
A. davidiana	Davidiana peach	10, 10, 10, 9, 9, 10, 10, 8, 0, 2, 10, 8, 0.

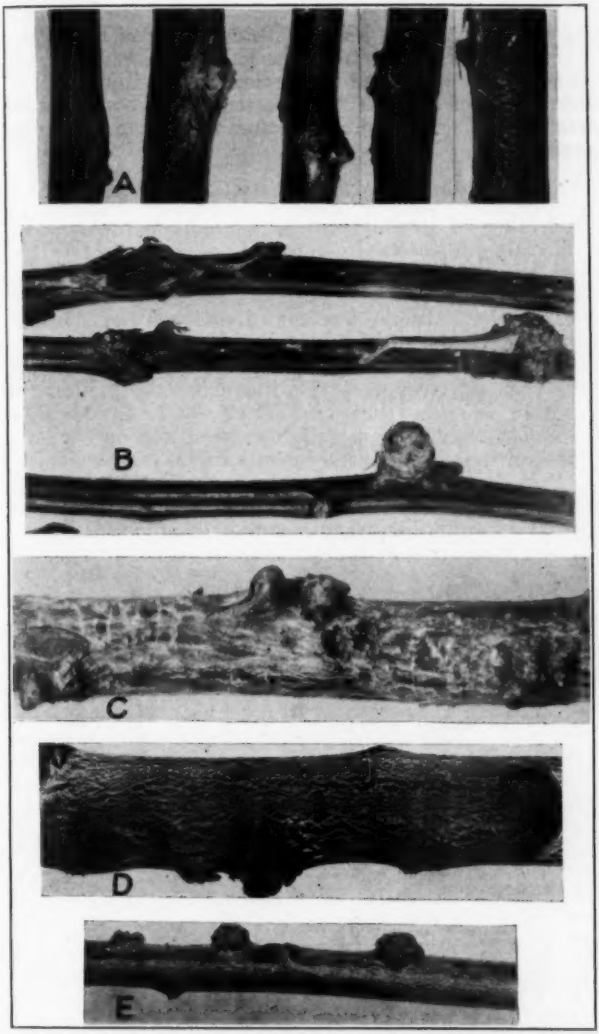
[a] Data do not include all the inoculations that were made.

Riker (*2*), experimenting with some inoculations on tomatoes, found that the size of the original puncture determined the size of the gall. The difference in size of the original puncture could hardly explain the relatively small size of the galls that developed on the resistant trees of *Prunus mume* as compared with the larger ones that appeared on the susceptible trees, since in all these inoculations a steel needle of the same size was used. The resulting wounds on



A.—Artificial inoculation on a susceptible variety of *Prunus mume* (S. P. I. 45523). The galls are typical crown-gall hypertrophies. July to December, 1922

B.—Artificial inoculations on a resistant variety of *Prunus mume* (S. P. I. 47950). Small pointlike hypertrophies are at the margins of the healing tissue. These hypertrophies, for the most part, do not increase in size. They are thought to be characteristic of resistance in Prunus. July to December, 1922



A.—Artificial hypertrophies on a resistant variety of *Prunus mume* (S. P. I. 47950). Enlarged about twice, to show small incipient galls. The puncture wounds have healed, but near their margins are these small pointlike growths. August to December, 1922

B.—Artificial galls on *P. armeniaca mandshurica*, to show form characteristic of a somewhat resistant variety of Prunus. The hypertrophies are developed at the margins and in one case at nearly right angles to surface of host. × 2. July to December, 1917

C.—Small hypertrophies on *Prunus armeniaca mandshurica*, to show small pointlike growths on margins of healing tissue. × 2. June, 1916, to March, 1917

D.—Small galls in *Prunus domestica* (Italian prune), to show nature of galls as they often develop. For illustrations of larger galls see (*5*). July to December, 1913

E.—Small galls on *Prunus domestica* (German prune). While these are typical galls in shape, their size is small. May to December, 1915

the resistant trees, as indicated by the scar of the healed injuries, were of a greater size than the galls that developed (pl. 2, B, and pl. 3, A). On the susceptible trees the galls for the most part correspond in size with the scar of the healed inoculation (pl. 2, A).

The formation of galls on this host can not always be correlated with growth activity. Negative results often take place on rapidly growing shoots.

SOIL INOCULATIONS BY GALL FRAGMENTS

The resistance of available nursery seedlings was tested in the manner already indicated, by inoculating the soil with fragments of galls at the time of planting. This method has been used by various investigators (7), and gave satisfactory results even before the true cause of the disease was known. It was employed as giving possibly more normal conditions of infection. It would seem to be at least a more natural method than puncture inoculation in the twigs. Small, one-year-old trees were also inoculated by gall fragments in the same way as were the germinating pits. In most instances a much smaller percentage of galls was produced on the small trees by this method than by artificial inoculation in the twigs of the same species. Probably some other factors may have been responsible for this difference. The inoculum must be rather variable in its pathogenicity when broken fragments of galls are used, although care was taken to collect fresh galls and use them very soon after they were broken up.

TABLE VII.—*Results after two years' growth from germinating pits planted with minced peach galls*

Stocks	Soil inoculated		Soil not inoculated	
	Number of trees	Number with galls	Number of trees	Number with galls
P. andersoni	17	1	6	1
Peach	30	28	32	1
Peach [a]	29	26	42	0
Peach-almond hybrid(?)	52	45	34	0
Almond (hard-shell)	57	36	42	1
Almond (bitter)	101	82	78	4
Almond (sweet)	98	79	42	1
Apricot (Royal)	46	22	42	0
Apricot [a]	42	16	25	2
P. fremonti	47	18	----	----
Almond (sweet)	71	49	----	----
Peach	47	25	----	----
Almond	35	25	----	----
A. davidiana	19	11	----	----
P. mume [b]	16	0	----	----
Almond [c]	16	10	----	----

[a] Treated with Bordeaux paste.
[b] Trees examined, but not dug up.
[c] As control on P. mume.

USES OF RESISTANT STOCK

A strongly resistant stock would have another use, in addition to that of propagating new trees. It could be used for inarching in orchards where the trees are already diseased with crown gall but not in too decadent condition. The inarching of small trees can

readily be performed without any great technical skill. A limited amount of experimental work in inarching Prunus into diseased apricot trees on peach stock has given very satisfactory results.

TABLE VIII.—*Results after two years' growth in soil inoculated with minced galls at the time of planting 1-year-old trees, 6 to 8 inches high*

Stock	Common name	Source [a]	Number of trees	Number with galls
P. cerasifera	Myrobalan	NS	139	4
P. cerasifera (hybrid)	Marianna	NS	155	7
P. insititia	St. Julian plum	NS	48	2
A. davidiana	Davidiana peach	USDA	41	35
P. americana	American plum	AA	56	2
P. munsoniana	Wildgoose plum	AA	18	0
P. besseyi	Sand cherry	NS	49	2
P. mume	Japanese apricot	USDA	16	0
P. domestica	P. D. stock [b]	NT	27	0
P. americana	Sprouts from old tree [c]		17	0

[a] The explanations of the letters used in Tables I, II, and VIII may be found under "Sources of material."

[b] Prunus domestica, a rootstock secured from sucker in a commercial orchard, and used to a limited extent in California.

[c] The sprouts of P. americana were secured from a tree (now dead) in the Botanical Garden, University of California.

GERMICIDES IN THE NURSERY

In the tests reported in Table VII certain of the germinating pits of apricot and peach were covered with a Bordeaux paste before planting. When this had become dried the pits were planted with and without minced galls. After two years no difference could be found between these trees and those growing in adjacent rows not treated with Bordeaux paste but inoculated with gall fragments.

Table IX is a summary of tests made by using different germicides on peach roots to protect them from infection with the gall organism when inoculated with fragments of peach galls. The peach roots were two years old, and none of them showed any evidence of crown gall when planted.

Slight injuries were made on the roots by light scraping before treatment with germicide. The gall fragments were mixed with the soil at the time of planting. The results indicate that under the conditions of the experiment the germicides were not very effective in preventing the development of crown gall, although in all tests but one there was a reduction in the percentage of galled trees. The conditions in all cases were much more favorable for infection than under usual orchard practice, in that there was abundant inoculum and injuries favorable for infection. All the germicides were more or less injurious to the roots, as was indicated by the number of trees dying as compared with the controls. The Bordeaux paste was no more efficient than the 12–15–50 Bordeaux mixture. These results are much less promising than those of Melhus and Manley (*1*), who, by using Bordeaux paste on bench-grafted apple roots, obtained very good results. The large amount of inoculum present in these tests of the writer may possibly explain the difference in results, and apples are possibly less susceptible than peaches

TABLE IX.—*Results from the treatment of 2-year-old peach roots with disinfecting chemicals, after which they were planted in soil infected with freshly broken-up crown galls and then grown for two years*

Treatment given	Number of trees dying	Number of trees with galls	Number of trees free from galls	Percentage of trees with galls
Bordeaux paste 3-6-4	14	23	14	62
Bordeaux mixture 4-6-50	6	20	4	83
Bordeaux mixture 12-15-50	12	7	11	38
Ortho lime-sulphur (concentrated)	5		All died.	
Ortho 1-10	2	14	9	60
Ortho 1-20	2	7	11	38
Ferrous sulphate (Bordeaux paste)[a]	7	3	18	14
Check	0	40	10	80

[a] This was made by the formula of J. W. Toumey, (7, *p. 26*): 1 part ferrous sulphate, 2 parts copper sulphate, and 3 parts quicklime.

SUMMARY

Forty different species (often several varieties per species) of Prunus and Amygdalus have been tested by making artificial inoculations with the crown-gall organism, *Pseudomonas tumefaciens*. There are apparently great differences in susceptibility among these species and often among varieties of the same species. This is especially true in such diversified species as *P. domestica*.

Resistance, from the data given sometimes seem to be a specific and at other times a varietal characteristic. In *Prunus domestica* some of the varieties tested have shown strong resistance, giving infection from 15 per cent or less of the inoculations. In the wild species *P. pumila*, *P. besseyi*, *P. mume*, *P. umbellata*, and *P. alleghaniensis*, the species as a whole is apparently strongly resistant. Further tests may, however, demonstrate that this resistance is a varietal characteristic.

The inoculation by punctures is a test more severe than would be found under natural conditions. The species in Table III probably would be completely resistant under nursery conditions. Possibly those showing less than 25 per cent of infection might also be classed as practically resistant.

Species of Amygdalus showed a high percentage of infection when the inoculations were made with gall fragments at the time of planting the pits. The one-year-old trees of various species inoculated by placing gall fragments around the roots showed a lower percentage of infection than those trees inoculated through punctures on the twigs.

Seven years of experimental inoculation on *Prunus mume* has demonstrated its high resistance. From 4,950 inoculations only 307 galls developed, or about 6 per cent of infection. Some of the introductions of this species are more susceptible than others; they show about 13 per cent of infection, which is comparatively low.

Preliminary inoculation tests on seedlings of *Prunus umbellata* indicate a considerable variation as regards susceptibility to crown gall (Table IV). Seven out of eleven of them gave less than 15 per cent infection. The results suggest the possibility of finding a strongly resistant strain of this species.

Amygdalus mira, the smooth-pit peach of China, has shown a very satisfactory resistance. Seedlings of this species are now being tested. *Amygdalus tangutica* and *A. persica potanini* are promising. Seedling peaches from commercial pits and standard commercial varieties are much more susceptible.

The Italian prune and other varieties of *P. domestica* (Table II) have shown resistance, and seedlings of these should be grown to test the inheritance of resistance.

Prunus armeniaca (S. P. I. 32834) and *P. armeniaca mandshurica* seem to show a higher resistance than the commercial varieties of apricot or their seedlings that have thus far been tested.

No practical method for using germicides in treating germinating pits or roots of the peach can as yet be recommended.

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GROWTH-EQUATION CONSTANTS IN CROP STUDIES [1]

By W. L. GAINES, *Chief in Milk Production*, and W. B. NEVENS, *Assistant Chief in Dairy Cattle Feeding, Agricultural Experiment Station, University of Illinois*

INTRODUCTION

The writers undertook, during the season of 1921, to obtain some experimental data as to the value of the sunflower crop for silage. There was at the time an increasing interest on the part of Illinois farmers in the sunflower as an alternative crop to corn for use as a silage crop. The investigation [2] was planned primarily for practical ends, and the proper stage of maturity for harvest seemed to be one of the questions on which information was needed.

The Mammoth Russian variety of sunflower was used, and the date of planting was May 18, 1921. The crop was grown at Urbana on a 10-acre field 80 rods long. The crop in three parts of the field was harvested for silage at three stages of maturity—87, 107, and 126 days after planting. The resulting silage was used in digestion and feeding trials in comparison with corn silage for milk production. This represented the limit of the facilities available for harvest and utilization of the crop as silage.

Further data as to the progressive changes in the crop with advance in maturity were obtained by quantitatively sampling the growing crop in the field at eight periods—65, 75, 86, 96, 106, 117, 127, and 138 days after planting. Four rows near the center of the field were reserved for this purpose. The plants for the field samples were selected in a systematic manner. The first plant for the sample at any period was taken at a preselected number from one end of the row. With this plant as a starting point, every hundredth plant was taken at the first sampling period, every ninety-ninth plant at the second period, and so on to every ninety-third plant at the eighth sampling period. The number in the row of the first plant selected for any sample was varied at each sampling period so as to insure some distance between the plants entering into the various samples, and thus avoid, in the samples, the effect of thinning by removal of plants. The plants selected at each period were cut 6 inches above ground level and the combined lot of 58 to 60 plants was at once weighed and subsampled for analysis. Separate determinations were made on stalk and seed, commencing 86 days after planting. Analysis included the analysis customarily made of feed: Dry matter, crude protein, crude fat, crude fiber, nitrogen-free extract, and ash. The

[1] Received for publication Apr. 22, 1925—issued January, 1926.

[2] More complete data than here given have been published in the following bulletins:

GAINES, W. L., and NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. COMPOSITION AND YIELD AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 268: 407-455, illus. 1925.

NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. FEEDING VALUE FOR DAIRY COWS; COMPOSITION AND DIGESTIBILITY WHEN ENSILED AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 253: 185-225, illus. 1924.

ash was also analyzed for aluminum, calcium, iron, magnesium, phosphorus, sodium, and sulphur. Computations have been made to acre yields on the basis of the number of plants per acre, 14,662. (The rows were 3.38 feet apart, and the plants averaged 10.55 inches apart in the row.)

While the investigation was not planned as a physiological study of plant growth, nevertheless the data accumulated shows something of the growth changes occurring in the sunflower crop. The curves given by the eight points of determination show, in the case of many of the crop constituents as analyzed, the general reverse curve characteristic of growth in annual plants and animals. Following the lead of Robertson,[3] particularly, the writers have utilized the general form of equation representing the course of an autocatalyzed monomolecular reaction in deriving a mathematical expression of the data from a growth standpoint. The results have seemed to the writers, rather suggestive as to the possibility of the constants of such growth equations serving a very useful purpose in crop studies. If the constants have the significance attached to them by Robertson, they should be of value in supplementing the data of final crop yield as customarily used in variety tests, etc. At least certain very striking differences appear in the constants as between species such as the sunflower and corn, as indicated by the data at hand. It is with the thought of somewhat emphasizing the differences thus shown that the present paper is offered.

THE AUTOCATALYTIC GROWTH CURVE

A brief review of Robertson's[4] presentation of the equations expressing the course of an autocatalytic monomolecular reaction will serve to bring out the background as to the significance to be attached to the constants of this sort of growth equation. Representing the amount of material at the start of such a reaction by A, and the amount transformed at any later time by x, then the amount of the original material left at any time is $A-x$. The velocity of the reaction is accelerated (catalyzed) by a product of the reaction present in amount proportional to x. The velocity is also proportional to the amount of original substance left, that is, $A-x$. Hence the velocity at any moment is proportional to $x(A-x)$. Designating time by t, the velocity is expressed as

$$\frac{dx}{dt}=kx(A-x) \qquad (1)$$

in which k is a specific constant.

It is obvious from (1) that the velocity will increase to a maximum with time until $x=A-x=\frac{1}{2}A$, after which time the velocity will continually decrease. Equation (1), when integrated, gives the relation

$$log\frac{x}{A-x}=K(t-t_1) \qquad (2)$$

in which $K=kA$, and $t_1=t$ when $x=A-x$. Time is thus given a negative value preceding the point at which A is one-half transformed, and a positive value thereafter.

[3] ROBERTSON, T. B. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. 1923.

[4] ROBERTSON, T. B. Op. cit.

Equation (2) is in form for application to observed data. The approximate application of the equation to growth data is comparatively simple. Figure 1 shows the general form of the curve. It has a lower asymptote of 0 and an upper asymptote of the value of A. If the growth observations are plotted as ordinates against time as abcsissas, a reasonable value can be chosen by inspection as representing the upper asymptote, and this value is taken as A. A line is then drawn at $\frac{1}{2}A$ and the point where the growth curve seems to cut this line is taken as t_1. Time may be taken from any point of origin and reckoned in any unit. In the present data the date of planting has been used as a convenient origin, and the day as a natural and convenient unit of time. For direct comparison between constants of different growth curves it is of course necessary that time be reckoned in the same unit. Having chosen values for

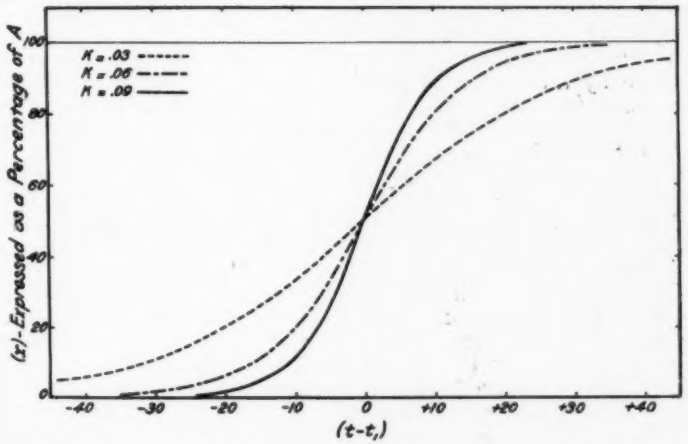

FIG. 1.—Showing form of the curve to the equation $\log \frac{x}{A-x} = K(t-t_1)$ for 3 values of K, 0.03, 0.06, and 0.09. (Note that a higher value of K means a shorter "grand period" of growth. The values of K in this paper lie within the range 0.03 to 0.09.)

A and t_1 the value of K required to satisfy each observation may be computed, and the average of the several values thus obtained is used as the value of K. Robertson's Table LX [5] facilitates the computations. The curve is then drawn, and if necessary slightly different values for the constants are chosen. The curve may be changed bodily to right or left by changing t_1. Its general slope may be increased or decreased by changing K. A better fit of the theoretical curves to the observed data may be obtained by correcting the constants as thus approximated by the method of least squares. For the present purpose the writers have considered this refinement as unnecessary.

The question, from the present standpoint, is, what significance may be attached to the constants of the equation when applied to the observational growth data. According to Robertson's view, A,

[5] ROBERTSON, T.B. Op. cit

or the final resultant of the growth processes, is to a considerable degree subject to modification by environmental conditions. On the other hand, k of equation (1) is a specific constant and represents certain inherent qualities of growth of the organism. In equation (2) $K=kA$, and hence after application to the observational data k of equation (1) may be estimated from A and K of equation (2) as $k=K/A$. According to this view K/A affords an index of genetic significance as representing the inherent rate or velocity of growth characteristic of the organism. Thus Robertson [6] finds that British male infants under the dissimilar environments of England and Australia have dissimilar values of A, namely 318.0 and 341.5, respectively, but similar values of K/A, namely, 0.000399 and 0.000398, respectively. The inference is that like heredity leads to like values of K/A, even though the environment is such as to lead to unlike values of A in the growth equations. If the constant has this genetic significance it affords a highly valuable measure of growth and inherent growth limitations.

In agricultural practice we are concerned with the final growth attained by the crop, and generally this is identical with A of the equation. In some cases, as will appear later, it is necessary to assume a higher value for A than that represented by the crop yield. It is apparent, therefore, that so far as affected by the value of A, the constant K/A varies in an inverse ratio to the crop yield. It will be seen in Figure 1 that a high value of K means a short-growing period, and quite naturally a short-growing period tends to be associated with a low-crop yield. Therefore the effect of K tends to give also an inverse ratio of the constant K/A to yield.

Conversely, A/K will tend to vary directly with crop yield, and consequently the constant in this form is somewhat better adapted for use in comparisons. Where K/A is a specific constant representing inherent growth *velocity* (that is, the rapidity with which full growth is accomplished, not the rate of absolute growth increase), A/K is a specific constant representing inherent final growth *capacity*. A/K is to be regarded, of course, simply as the reciprocal of k of equation (1). Its value as an index of growth capacity or crop yield depends upon the association between the length of time that the crop or crop constituent under consideration continues to grow and the final extent of growth. Experience indicates that length of growing period and extent of growth or crop yield are directly associated. On this reasoning the constant A/K should represent the inherent capacity for crop yield as between genetically different varieties or species under optimum or comparable conditions of environment. How well it may actually serve such a scientifically important and practically useful purpose remains to be demonstrated by critical experiment.

The data of the present paper pertain to the sunflower crop and the corn crop, the data for corn being derived from the work of Jones and Huston.[7] The data pertain not to individual plants directly but to acre populations collectively. In the graphic presentation the same scale is used for time in days throughout, but the scale

[6] ROBERTSON, T. B. Op. cit. p. 40.

[7] JONES, W. J. and HUSTON, H. A. COMPOSITION OF MAIZE AT VARIOUS STAGES OF ITS GROWTH. Ind. Agr. Exp. Sta. Bul. 175: 599-630, illus. 1914.

for growth in pounds is varied, being expressed uniformly as a percentage of the theoretical total or final growth. The sunflower equations may be converted to the average individual plant (upwards of 6 inches above ground) by dividing A by 14,662. The corn equations may be converted to the average individual plant (above ground) by dividing A by 10,000. Just what effect this difference in density of the population may have caused in the constants of the sunflower and corn equations the writers have no means of knowing precisely. Theoretically, A/K should not be affected by this or other environmental factors. At the present stage, of course, the whole theoretical matter may be regarded as no more than a working hypothesis.

CRUDE FIBER AND ALUMINUM IN THE SUNFLOWER

Crude fiber is a constituent of the plant customarily determined in feed analyses. It consists principally of the cellulose tissues of the plant. This constituent corresponds in a way to the skeleton of an

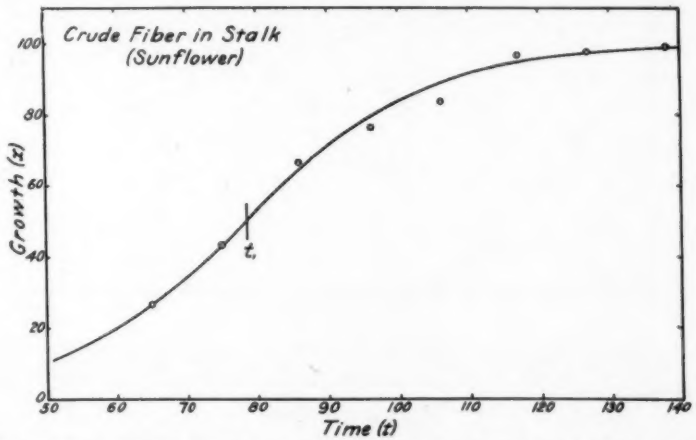

FIG. 2.—Growth of crude fiber in stalk per acre of sunflower crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 2,700 pounds. Equation to curve:

$$\log \frac{x}{2700-x} = 0.033\ (t-78.5)$$

animal, and affords a good measure of growth in size or volume of the plant. The observations and fitted curve for the crude fiber in the stalk [8] are shown in Figure 2. The curve gives a fair fit to the observations, although a considerably better fit is obtained by a logical modification, as shown in Figure 4. The curve is presented in the form given in Figure 2 partly for the sake of comparison with Figure 3, which pertains to aluminum.

The observations and fitted curve for aluminum are shown in Figure 3. Considerable irregularity is manifest in the aluminum

[8] The seeds were separated carefully by hand, and the stalk includes the remainder of the head and plant as cut 6 inches above ground. In the case of the corn the ears (that is, cob and kernels together) were separated, and the stalk includes the remainder of the ear (husk and shank) and plant as cut at ground level.

data. This irregularity may be partly attributable to difficulty of chemical determination.

Crude fiber and aluminum are unique in that they are the only constituents of the stalk, as determined, that do not undergo more or less marked depletion in the stalk coincident with the development of the seed. This suggests that the two are in some way associated. It is found from the equations given that K is the same in both cases, but that t_1 falls 18.5 days later in the aluminum curve than it does in the crude-fiber curve. If the aluminum curve is shifted 18.5 days to the left, and superimposed on the crude fiber curve, the two curves coincide. Aluminum is thus directly proportional to the crude fiber of 18.5 days preceding. If aluminum is an essential element in the building up of the crude-fiber tissues, its accumulation should occur

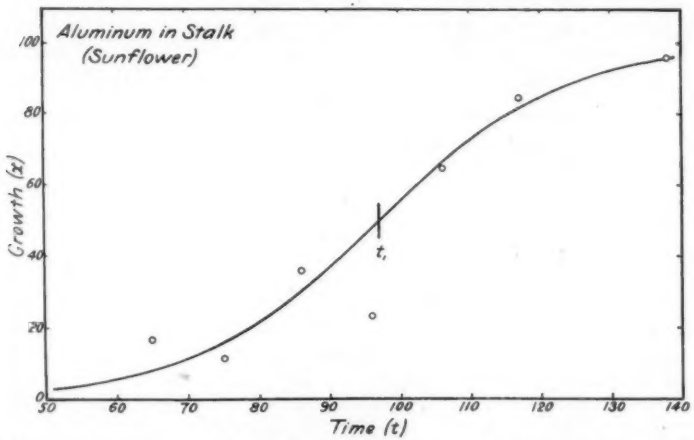

FIG. 3.—Growth of aluminum in stalk per acre of sunflower crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 7.5 pounds. One observation at $t=127$ and $x=145$ (=10.86 pounds per acre) is not included in the graph and was ignored in fitting the curve Equation to curve:

$$\log \frac{x}{7.5-x}=0.033\ (t-97)$$

simultaneously with or precede that of the crude fiber. On the other hand, if the aluminum represents a nonessential constituent held mechanically by the crude fiber structures of the plant, it might be expected to accumulate in proportion to the crude fiber, but at a later time. The pronounced lag in the appearance of aluminum favors the view that it is associated with the crude fiber as a mere mechanical accumulation, rather than as an essential element of the plant tissues. This illustrates one use of the growth-equation constants, and brings out a relation which might otherwise easily escape detection.

CRUDE FIBER IN STALK OF SUNFLOWER AND CORN

The vegetative and reproductive stages of growth of annual plants represent two distinct aspects of the physiology of growth of the plant as a whole. The present data pertaining to the stalk principally

represent the vegetative phase, but they also include certain portions of the plant directly concerned with the reproductive phase. It is reasonable, therefore, to treat the data as representing two cycles of growth. Such treatment is suggested also by the fact that growth in animals in many cases occurs in two or three distinct cycles.

In Figure 4, the observational data for crude fiber in the stalk of the sunflower are repeated. Two curves of growth (designated x' and x'') are given, together with the sum of these two as representing the observed data. It will be observed at once from Figure 4 that the theoretical curve as thus derived agrees better with the observations than does the single-cycle curve of Figure 2. The root mean-square error for the single-cycle curve is 66 pounds, and for the double-cycle curve 40 pounds.

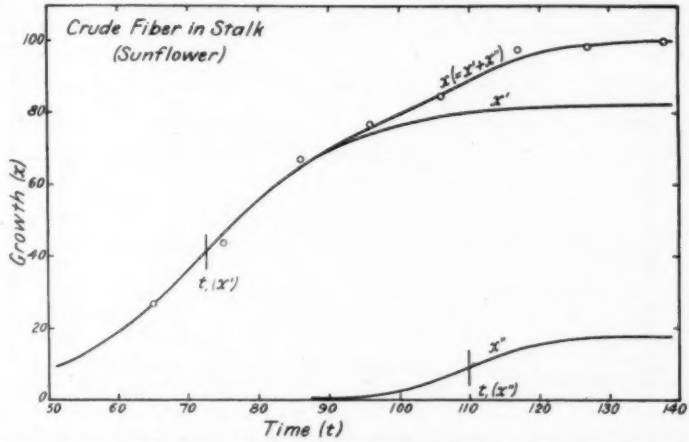

FIG. 4.—Growth of crude fiber in stalk per acre of sunflower crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 2,680 pounds. Observations same as Figure 2, treated as a two-cycle phenomenon. Equations to curves:

$$\log \frac{x'}{2200-x'} = 0.042\ (t-72.5)$$

$$\log \frac{x''}{480-x''} = 0.082\ (t-110)$$

Reed and Holland [9] studied the growth of the sunflower plant in height (cm.) and from their data derived the equation

$$\log \frac{x}{254.5-x} = .042\ (t-34.2)$$

time being counted in days from a date following planting not definitely stated. K of their equation agrees exactly with K for the first cycle equation of Figure 4, a value considerably different than K of the single-cycle curve of Figure 2. Since growth in height of the plant is not accelerated by seed development, the relation between the K's of the three equations may be taken as further evidence that the crude-fiber data represent a two-cycle growth process.

[9] REED, H. S., and HOLLAND, R. H. THE GROWTH RATE OF AN ANNUAL PLANT HELIANTHUS. Proc. Nat. Acad. Sci. 5: 135-144, illus. 1919.

The data for crude fiber in the stalk of corn are presented in Figure 5. Mere inspection of the plotted observations is sufficient to show that they do not conform to the form of curve exhibited by the general equation as illustrated in Figure 1. Treatment of the data, however, as a two-cycle matter yields a theoretical curve that conforms beautifully to the observations. Just how well it may satisfy the reason is perhaps another matter. It involves the assumption that the second growth cycle is at its maximum velocity at the time of the last observation. This assumption, it will be apparent, attaches great weight to the accuracy of the last observational value as representing the course of growth, and should be supported by other experimental work before full acceptance.

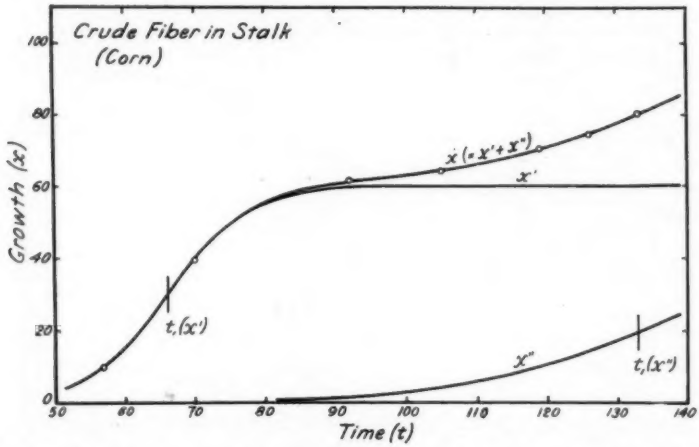

FIG. 5.—Growth of crude fiber in stalk per acre of corn crop. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 1,710 pounds. Equations to curves:

$$\log \frac{x'}{1030-x'} = 0.075\ (t-66.5)$$

$$\log \frac{x''}{680-x''} = 0.033\ (t-133)$$

Taken at face value, the data of Figure 5 indicate that the corn is potentially capable of a much greater growth in the reproductive stage than was actually realized, as far as the limitation of the tissues, represented by the present crude fiber data, is concerned. [There is a different relation for the crude-fiber data of the ear (fig. 9).] It is apparent from the data of Figures 4 and 5, that the crude-fiber growth curves of the sunflower and corn are distinctly different. In the first or vegetative cycle the sunflower has a low value of K (0.042) and the corn has a high value (0.075). In the second or reproductive cycle these relations are reversed—that is, the sunflower has a short "grand period" of growth ($K=0.082$) and the corn has a long one ($K=0.033$).

As to the particular location of the growth in crude fiber in the stalk in the second cycle, the data of Figures 4 and 5 do not distinguish. Presumably, the location is in part, at least, in those

organs directly associated with seed development, but included in the stalk data. For the sunflower this would be the head except the seed, and for the corn the husk and shank. Fortunately some data are available in the case of corn showing the proportion of crude fiber in the husk and the remainder of the stalk. Schweitzer [10] gives data which indicate that 19 per cent of the crude fiber of the stalk 138 days after planting was in the husk. Grindley [11] found in a crop of corn yielding 100 bushels per acre that 18 per cent of the crude fiber of the stalk was in the husk and silk. According to the equations of Figure 5, at 133 days after planting the second cycle is responsible for 24.8 per cent of the crude fiber of the stalk. Bearing in mind that the shank is not included in the proportions of 19 and 18 per cent, as estimated from Schweitzer's and Grindley's figures, the proportions suggested in Figure 5 from the theoretical treatment of the combined data are consistent with the view that the second growth cycle indicated by the stalk data is quite directly associated with seed development.

GROWTH IN SEED AND EAR

The data just examined indicate that growth in the sunflower and corn crops occurs in two cycles, that the first cycle is associated with the vegetative functions and the second with the reproductive functions. In agreement with this, the development of seed and ear seems to constitute a well defined growth cycle, which, however, in the case of certain constituents is not completed at the death of the stalk. The writers' data on the sunflower seed show that all of the constituents except nitrogen-free extract continued to increase in substantial conformity with the autocatalytic formula. This is in contrast with the behavior of the same constituents of the stalk. In the stalk there is first an accumulation and then a loss in the case of all constituents except crude fiber and aluminum. This loss may be regarded as due to a process of senescence, the reverse of growth.

Properly, one might regard the development of the seed as cycle one, and accordingly A of the seed growth equations is to be divided by the number of seeds per acre to convert the equation to an individual basis. Another growth cycle then ensues at that later time when the seed is provided with the conditions for germination and growth. This cycle also conforms to the autocatalytic formula with reference to the essentially structural materials. With reference to other materials, however, the processes of senescence predominate over those of growth after a time, so that the equation used is not alone adequate to describe the entire changes.

Robertson [12] has analyzed data of Monnier on the growth of the oats plant and has reached the conclusion that the mineral constituents did not follow the autocatalytic law of growth. Considering the sunflower crop as a whole, the writers' data would require the same conclusion with reference not only to the mineral constituents but also to all the other constituents, except crude fiber, crude fat, and aluminum. This condition is due, however, to the senile losses in

[10] SCHWEITZER, P. STUDY OF THE LIFE HISTORY OF CORN AT ITS DIFFERENT PERIODS OF GROWTH. Mo. Agr. Exp. Sta. Bul. 9, 78 p. 1889.
[11] Unpublished data, Ill. Agr. Exp. Sta.
[12] ROBERTSON, T. B. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. 1923.

the stalk. Growth of ash in the seed conforms very well to the formula, and this is true also for the several elements of the ash.

Some interesting comparisons may be made between the growth curves of the various constituents of the seed. Phosphorus and protein (nitrogen × 6.25) have the same value of K, and protein is directly proportional to the phosphorus of 7 days preceding. The curves for sulphur and fat also have some similarity, in that t_1 is 108 for each, a considerably delayed value as compared with other constituents; and also, for each it is necessary to assume that the growth process was still in active progress at the last observation. The curves for all the data have been worked out to a first approximation,[13] but there is no need to repeat all of them here. The data for dry matter and crude fiber in seed of sunflower and ear of corn are given, however, for the purpose of certain further comparisons of the two crops on the basis of the growth constants.

DRY MATTER IN SEED AND EAR

The percentage water content of plant tissues varies widely with maturity of the plant. Thus at the time of the first observation on the sunflower seed the water content was 86 per cent and at the last

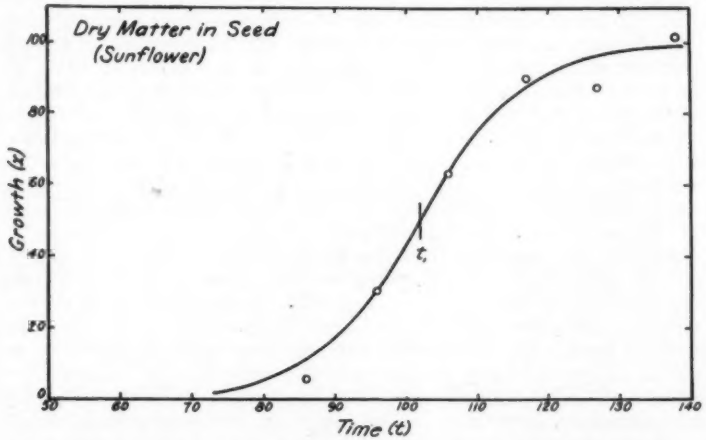

FIG. 6.—Growth of dry matter in seed of sunflower per acre. Time (t) is reckoned in days from date planting. Growth (x) is expressed as a percentage of 1,450 pounds. Equation to curve:

$$\log \frac{x}{1450-x} = 0.058\ (t-102)$$

observation 15 per cent. There are obvious reasons for dealing with the dry matter as a measure of growth. The data for the seed of sunflower and ear of corn are presented in Figures 6 and 7, respectively. The course of growth, as measured by dry matter, conforms satisfactorily to the theoretical considerations. Considering the equations, we find the sunflower with a large value of K (0.058)

[13] GAINES, W. L., and NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. COMPOSITION AND YIELD AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 268: 407–455, illus. 1925

and the corn with a small value of K (0.038), this agreeing in a general way with the hint offered by the second growth cycle of the stalk as the data were treated in Figures 4 and 5. Another difference in the curves is notable: In treating the growth in dry matter of the ear by the method used it is necessary to assume that growth had not reached its natural limit at the last observation. Such an assumption is not necessary for the sunflower data. This again is consistent with the treatment of the corresponding data of Figures 4 and 5.

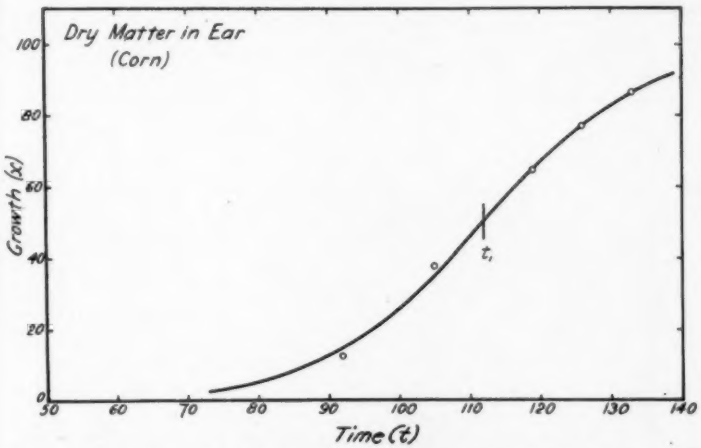

FIG. 7.—Growth of dry matter in ear of corn per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 6,000 pounds. Equation to curve:

$$\log \frac{x}{6000-x} = 0.038\,(t-112)$$

CRUDE FIBER IN SEED AND EAR

The course of development of crude fiber in the seed of sunflower (fig. 8) is practically identical with that of dry matter. This is partly a consequence of the fact that crude fiber constitutes a high and fairly constant proportion of the dry matter of the seed throughout the growth cycle. The per cent of crude fiber based on dry matter lies within the range 31 to 39.

The course of crude-fiber development in the ear of corn (fig. 9), however, presents a contrast to that of dry matter. The crude fiber of the ear is largely in the cob (about 85 per cent at maturity). Figure 9 may be presumed, therefore, to represent approximately the growth of the cob. On the basis of interpretation by the equation, the crude-fiber growth cycle is completed, whereas it is not completed in case of dry matter. The apparent completion of the growth cycle of the cob contrasts also with the incomplete second cycle of Figure 5, taken to represent growth of husk and shank. If one were to speculate he might infer that the inherent growth limitations of the cob are a limiting factor in the maximum growth

of the ear. It would seem that any study of the growth of corn from the present standpoint should take account of the growth of the cob as a unit by itself.

GROWTH-CAPACITY CONSTANTS OF SUNFLOWER AND CORN

As a final comparison between the sunflower and corn equations, we may consider the growth-capacity constant A/K. As previously discussed, this constant is theoretically specifically related to the inherent or genetic characteristics of the organism. Since the formula seems to apply equally well to each of several constituents of the crop,

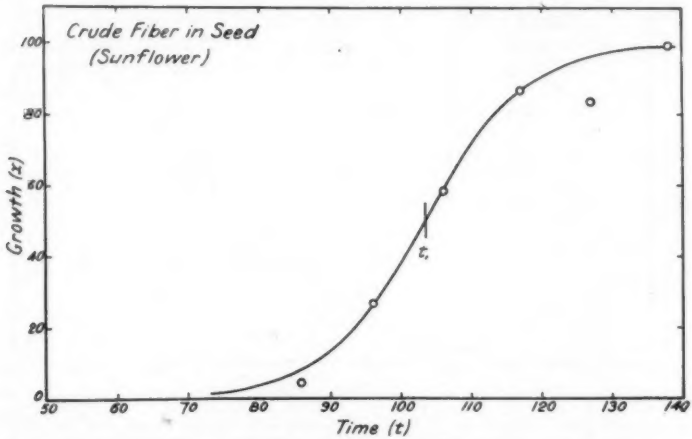

FIG. 8.—Growth of crude fiber in seed of sunflower per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 580 pounds. Equation to curve:

$$\log \frac{x}{580-x} = 0.060\,(t-103.5)$$

it may be presumed that the A/K constant is of equal significance for the several constituents. Table I gives the values of the growth capacity constants.

TABLE I.—*Comparison of the growth capacity constants of certain constituents of the sunflower and corn crops*

Constituent	$A/K \times 10^{-2}$	
	Sunflower	Corn
Crude fiber in stalk [a]	440	165
Crude fiber in seed or ear	97	74
Dry matter in seed or ear	250	1,579

[a] Weighted average of first and second cycles

From this table it appears that the sunflower is preeminently a crude-fiber or roughage crop, while corn is preeminently a grain crop. Comparing crude fiber in the stalk, the sunflower has greater

growth capacity, the constant being 2.7 times that of corn. Comparing dry matter in seed and ear, corn has much the greater capacity, the constant being 6.3 times that of the sunflower. Or we may compare the ratio of dry matter in seed or ear to crude fiber in stalk and find that the ratio for corn is 17 times the ratio for the sunflower. As a cultivated crop, corn is evidently much better adapted to the production of maximum yields of valuable food or feed stuffs than is the sunflower. Breeding and selection may serve to improve the sunflower, but with its apparent inherent handicap of high vegetative and low reproductive development, it has little prospect of ever being a competitor of corn under conditions which permit the growing of corn.

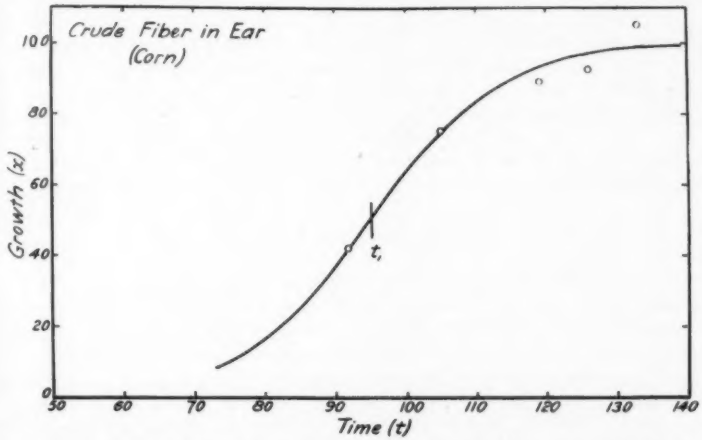

FIG. 9.—Growth of crude fiber in ear of corn per acre. Time (t) is reckoned in days from date of planting. Growth (x) is expressed as a percentage of 350 pounds. Equation to curve:

$$\log \frac{x}{350-x} = 0.047\ (t-95)$$

SUMMARY

Data on the growth of the sunflower [11] and of corn from Bulletin 175, Indiana Agricultural Experiment Station are analyzed (following Robertson [15]) by application of the formula $\log \frac{x}{A-x} = K\ (t-t_1)$, in which x represents growth accomplished at any time, t. The constants A, K, and A/K of this equation are regarded as of definite significance in connection with crop studies. The constants serve to give numerical expression to the course of growth changes in the crop and should be of value in supplementing the usual data of final crop yield. As between the sunflower and corn crop data studied, pronounced difference is shown, particularly with reference to the vegetative and reproductive growth cycles.

[11] GAINES, W. L., and NEVENS, W. B. THE SUNFLOWER AS A SILAGE CROP. COMPOSITION AND YIELD AT DIFFERENT STAGES OF MATURITY. Ill. Agr. Exp. Sta. Bul. 268: 407–455, illus. 1925.

[15] JONES, W. J., and HUSTON, H. A. COMPOSITION OF MAIZE AT VARIOUS STAGES OF ITS GROWTH. Ind. Agr. Exp. Sta. Bul. 175: 599–630, illus. 1914.

ROBERTSON, T. B. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. 1923.

NET-ENERGY VALUES OF ALFALFA HAY AND ALFALFA MEAL[1]

By E. B. FORBES, *Director*, J. AUGUST FRIES, *Assistant Director*, and W. W. BRAMAN, *Associate, Institute of Animal Nutrition, of Pennsylvania State College*

INTRODUCTION

The investigation reported here was undertaken to determine the effect of fine grinding of alfalfa hay, as in the preparation of commercial alfalfa meal, on its net-energy value. Some of the results, computed by an older method, were incorporated in an earlier publication,[2] but the detailed account of the experiment is first presented in detail here.

The subject of the experiment was a purebred Shorthorn steer 20 months old and having an initial weight of 345 kilograms.

The general plan of the experiment was to feed to this steer, alternately, alfalfa hay and alfalfa meal during six periods (a pair of rations on each of three planes of intake).

The method of experimentation was the same as in the previous net-energy determinations at this institute by the use of the respiration calorimeter.[3]

PERIODS AND RATIONS

Each feeding period extended over 21 days, of which the first 11 were preliminary and the last 10 the period of the digestion and energy metabolism experiment. On the eighteenth and nineteenth days, 48-hour determinations of the respiration products and the heat production were made by means of the respiration calorimeter. The dates of the several periods, the rations fed, and the average live weight of the animal in each period are shown in Table I.

TABLE I.—*Periods, rations, and average live weights*

Period	Preliminary period, 1911–12 [a]	Digestion period, 1912	Alfalfa hay	Alfalfa meal	Live weights [b]
			Kg.	*Kg.*	*Kg.*
I	Dec. 24–Jan. 3	Jan. 4–13	7.5		348.6
II	Jan. 14–24	Jan. 25–Feb. 3		7.5	348.7
III	Feb. 4–14	Feb. 15–24	6.0		353.7
IV	Feb. 25–Mar. 6	Mar. 7–16		6.0	349.4
V	Mar. 17–27	Mar. 28–Apr. 6	3.5		337.1
VI	Apr. 7–17	Apr. 18–27		3.5	329.4

[a] All dates are inclusive.

[b] Average of last 6 weights taken at 8 a. m.

[1] Received for publication Apr. 10, 1925; issued January, 1926. This investigation was conducted with the financial cooperation of the Bureau of Animal Industry of the U. S. Department of Agriculture. It was planned, in detail, by H. P. Armsby, and executed by J. A. Fries, W. W. Braman, D. C. Cochrane, K. K. Jones, F. W. Christensen, J. W. Parke, and F. C. Dosé.

[2] ARMSBY, H. P., and FRIES, J. A. NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. Jour. Agr. Research 3: 435–491, illus. 1915.

[3] ARMSBY, H. P., and FRIES, J. A. Op. cit.

———, and FRIES, J. A. THE INFLUENCE OF TYPE AND OF AGE UPON THE UTILIZATION OF FEED BY CATTLE. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 128, 245 p., illus. 1911.

PREPARATION OF THE ALFALFA HAY AND ALFALFA MEAL

The alfalfa hay used was grown in Idaho. From a chosen lot, one half was ground to a fine meal, in the presence of H. P. Armsby, on November 20, 1911, at a commercial mill in Kansas City, Mo. The fine dust which separated from the hay during the grinding was carefully collected and returned to the ground hay. Then both the ground and the unground hay were shipped to the Institute of Animal Nutrition at State College, Pa. The hay was of a bright-green color and excellent quality.

In order to reduce the unground hay to a condition making it convenient to sample for chemical analysis and to handle in the metabolism experiment, it was cut by running through a silage cutter (without the blower). During this process much of the leaf broke up into a fine condition. Of this cut hay, 60 per cent would pass through a $\frac{3}{16}$-inch-mesh sieve, and about 35 per cent through a $\frac{1}{8}$-inch-mesh sieve. Comparatively long pieces, however, passed through these sieves lengthwise.

On account of the light, dusty character of the finely ground hay it was necessary to feed it mixed with water, which was added in an amount about equal to the weight of the hay; and in order to keep conditions as nearly as possible the same in all periods, the coarsely cut hay was similarly moistened.

COMPOSITION OF THE FEEDING STUFFS

The composition and energy values of dry matter of the feeds are given in Table II. True protein, as there reported, was determined by the Stutzer method.

TABLE II.—*Dry matter of alfalfa hay and alfalfa meal, and composition of the dry matter*

	Hay				Meal			
	Period I	Period III	Period V	Average	Period II	Period IV	Period VI	Average
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Dry matter	88.52	88.67	87.21	88.13	88.98	90.13	90.14	89.75
Ash	9.14	8.93	9.12	9.06	9.54	9.09	9.09	9.24
True protein	12.70	12.29	12.13	12.37	12.06	11.60	11.60	11.75
Nonprotein	2.63	3.05	2.91	2.86	2.82	2.89	2.89	2.87
Crude fiber	29.44	30.06	30.82	30.11	30.49	31.44	31.44	31.12
Nitrogen-free extract	44.10	43.70	43.11	43.64	43.37	43.07	43.07	43.17
Ether extract	1.99	1.97	1.91	1.96	1.72	1.91	1.91	1.85
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Total nitrogen	2.59	2.61	2.56	2.59	2.53	2.47	2.47	2.49
True protein nitrogen	2.03	1.97	1.94	1.98	1.93	1.86	1.86	1.88
Carbon	44.89	44.78	45.27	44.98	44.92	45.17	45.17	45.09
Energy, calories * per kg	4,353.80	4,338.30	4,411.80	4,368.00	4,363.70	4,378.70	4,378.70	4,373.70

* Throughout this article the word "calorie" signifies the large, or kilogram, calorie, unless the contrary is specifically stated.

DIGESTIBILITY

The digestibility of the ration was determined in the usual manner. Table III sets forth the digestion coefficients of the hay and of the meal.

TABLE III.—*Digestion coefficients of alfalfa hay and alfalfa meal*

Feed and period	Dry matter	Organic matter	True protein	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Carbon	Energy
Alfalfa hay, dry matter:									
Period I, 6,638.3 grams...	59.9	62.2	71.4	76.6	40.6	74.0	14.4	58.7	58.8
Period III, 5,320.2 grams.	60.4	61.7	67.7	74.1	45.0	70.7	19.4	59.0	58.6
Period V, 3,052.4 grams..	63.7	64.4	71.0	74.4	47.3	73.6	35.2	63.0	62.3
Average	61.3	62.8	70.0	75.0	44.3	72.8	23.0	60.2	59.9
Alfalfa meal, dry matter:									
Period II, 6,670.7 grams..	58.2	59.4	66.1	72.7	38.0	72.2	2.4	57.5	56.8
Period IV, 5,407.8 grams.	57.8	59.5	66.3	72.5	37.2	72.5	31.1	56.5	56.6
Period VI, 3,154.9 grams.	61.3	61.7	69.3	75.8	43.1	71.9	35.1	60.5	60.5
Average	59.1	60.2	67.2	73.7	39.4	72.2	22.9	58.2	58.0

In all periods the hay was appreciably more completely digested than was the meal. This difference prevailed consistently, without exception, in regard to dry matter, organic matter, true protein, crude protein, and crude fiber. There were exceptions to this order, in some cases, with reference to nitrogen-free extract and ether extract. Naturally these differences in the digestibility of alfalfa hay and alfalfa meal depended on the meal being used as the sole roughage.

While the writers do not have positive evidence to explain this difference, it seems proper to suggest a probable explanation. In view of their understanding of the conditions which determine the course of the food in the alimentary tract of ruminants, especially the influence of fineness of grinding on the passage of the food when swallowed, into the first, second, and third stomachs, and its tendency therefore, to suppress rumination, it seems likely to the writers that the lower digestibility of the meal was due to its having been swallowed, in part at least, past the paunch, and, consequently, to having escaped to this extent the usual prolonged soaking and fermentation in that organ, and the subsequent regurgitation and remastication, while normal conditions prevailed in the case of the coarsely cut hay.

The higher apparent digestibility of the hay and the meal in Periods V and VI than in Periods I to IV is not unusual in periods of of very low feed intake, this being due in part to the slower passage of the feed through the alimentary tract and increased fermentation per unit of weight of feed. In these periods of low feed intake there was also evidence of slight abnormality in the condition of the subject, perhaps as a result of slow movement of alimentary residues, the indications being irregular drinking, abundant sediment in the urine, mucus in the feces, and foul odor of urine and feces. The daily fecal elimination, however, was not unduly variable, not sufficiently so to warrant a conclusion that fecal elimination was so delayed as seriously to compromise the accuracy of the digestion coefficients.

THE BALANCE OF MATTER

From the data for balance of matter, in Table IV, it will be noted that with decreased feed intake there was marked decrease in methane elimination, but when we relate the amount of the methane to the amount of the feed we see that with decrease in feed intake there was a slight increase in the methane production per kilogram of dry matter of feed. Thus in Periods I, III, and V, with feed intake of 6,638, 5,320, and 3,052 grams, respectively, the methane production per kilogram of dry matter of feed was 18.769, 19.453, and 21.871 grams, respectively.

TABLE IV.—*Balance of matter and energy per day and head*

	Period I					Period III				
	Dry matter	Water	Nitrogen	Carbon	Energy	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*
Alfalfa hay	6,638.3	6,332.0	172.0	2,980.2	28,921.1	5,320.2	4,675.0	139.1	2,382.4	23,178.5
Water		13,295.0					16,100.0			
Total	6,638.3	19,627.0	172.0	2,980.2	28,921.1	5,320.2	20,775.0	139.1	2,382.4	23,178.5
Outgo, Group I:										
Feces	2,663.2	9,710.1	40.2	1,230.9	11,924.3	2,104.8	9,493.0	36.1	977.5	9,601.8
Urine	723.0	8,316.3	115.2	166.8	[a]1,819.5	561.6	5,366.2	93.7	137.5	[a]1,420.1
Methane	137.95			103.2	1,840.8	112.9			84.5	1,506.5
Total					15,584.6					12,528.4
Metabolizable					13,336.5					10,650.1
Outgo, Group II:										
Hair and brushings	12.7	1.1	1.0	5.5	60.7	12.7	1.1		5.5	60.7
Carbon dioxide	4,719.0			1,286.9		4,054.0				
Water vapor		4,587.5					3,740.2			
Body balances:										
Water		−2,988.0					+2,174.5			
Protein	+93.0		+15.5	+48.9	[a]+414.6	+49.8		+8.3	+26.2	[a]+221.1
Fat	+180.5			+138.0	+1,714.3	+59.7			+45.7	+567.2
Computed heat					11,146.9					9,801.1
Observed heat					11,088.5					9,723.3

	Period V					Period II				
	Dry matter	Water	Nitrogen	Carbon	Energy	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*
Alfalfa hay	3,052.4	4,458.0	78.1	1,381.8	13,298.4	[b]6,670.7	7,846.0	168.7	2,996.7	29,175.6
Water		6,975.0					16,240.0			
Total	3,052.4	11,433.0	78.1	1,381.8	13,298.4	6,670.7	24,086.0	168.7	2,996.7	29,175.6
Outgo, Group I:										
Feces	1,108.5	4,938.0	20.0	511.3	5,010.4	2,786.9	11,798.0	46.0	1,273.0	12,600.0
Urine	362.1	4,117.6	65.3	98.1	[a]865.2	677.3	7,357.5	107.1	161.5	[a]1,666.6
Methane	68.4			51.2	912.8	125.2			93.7	1,670.7
Total					6,788.4					15,937.3
Metabolizable					6,510.0					13,238.3
Outgo, Group III:										
Hair and brushings	12.7	1.1	1.0	5.5	60.7	12.7	1.1	1.0	5.5	60.7
Carbon dioxide	2,850.8			777.4		4,593.3			1,252.6	
Water vapor		2,509.1					4,547.1			
Body balances:										
Water		−132.8					+382.3			
Protein	−49.2		−8.2	−25.8	[a]−219.3	+87.6		+14.6	+46.0	[a]+390.5
Fat	−46.9			−35.9	−445.6	+215.0			+164.4	+2,042.1
Computed heat					7,114.2					10,745.0
Observed heat					7,303.7					10,886.0

[a] Corrected to nitrogen equilibrium.

[b] Alfalfa meal.

TABLE IV.—*Balance of matter and energy per day and head*—Continued

	Period IV					Period VI				
	Dry matter	Water	Nitrogen	Carbon	Energy	Dry matter	Water	Nitrogen	Carbon	Energy
Income:	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*	*Grams*	*Grams*	*Grams*	*Grams*	*Calories*
Alfalfa meal	5,407.8	4,607.0	133.6	2,442.8	23,652.1	3,154.9	4,346.0	78.0	1,425.1	13,798.6
Water		12,000.0					4,250.0			
Total	5,407.8	16,607.0	133.6	2,442.8	23,652.1	3,154.9	8,596.0	78.0	1,425.1	13,798.6
Outgo, Group I:										
Feces	2,280.5	9,168.0	36.8	1,062.8	10,255.5	1,221.3	4,583.0	18.9	563.3	5,444.8
Urine	524.5	5,804.4	90.7	131.4	[a]1,331.6	288.1	3,752.3	58.3	102.6	[a]846.7
Methane	105.2			78.7	1,403.8	69.0			51.6	920.1
Total					12,990.9					7,211.6
Metabolizable					10,661.2					6,587.0
Outgo, Group II:										
Hair and brushings	12.7	1.1	1.0	5.5	60.7	12.7	1.1	1.0	5.5	60.7
Carbon dioxide	4,004.1			1,092.0		2,694.7			734.9	
Water vapor		3,791.5					2,391.9			
Body balances:										
Water		−2,158.0					−2,132.3			
Protein	+30.6		+5.1	+16.1	[a]+136.4	−1.2		0.2	0.6	[a]−5.3
Fat	+73.7			+56.3	+699.7	−42.1			32.2	−399.5
Computed heat					9,764.4					6,931.1
Observed heat					9,697.6					6,795.0

[a] Corrected to nitrogen equipment.

The balances of protein and fat reflect the differing intake of feed in a generally consistent manner, but the losses of protein in Periods V and VI are not as nearly alike as might have been anticipated.

The data representing the balance of energy in the several periods are presented, for reference, in Table IV, but the significance of the figures, in comparison, is more readily apparant in another arrangement, and will be discussed in connection with Table VII.

HEAT PRODUCTION

The observed heat production (Table V) is the measured heat emission corrected for gain or loss of actual heat in body gain or loss and for loss of heat in excreta.

TABLE V.—*Observed and computed average daily heat production*

	Observed heat	Computed heat	Apparent error	Computed, as per cent of observed
	Calories	*Calories*	*Calories*	
Period I	11,088.5	11,146.9	+58.4	100.5
Period III	9,723.3	9,801.1	+77.8	100.8
Period V	7,303.7	7,114.2	−189.5	97.4
Period II	10,886.0	10,745.0	−141.0	98.7
Period IV	9,697.6	9,764.4	+66.8	100.7
Period VI	6,795.0	6,931.1	+136.1	102.0

The accuracy of this observed heat production may be confirmed by indirect computation—subtracting from the gross energy of the feed the determined energy of the excreta (urine, feces, and combustible gases), and the energy of the body gains, as computed from the balances of carbon and nitrogen. Such a comparison of

the observed and the computed heat production is made in Table V and shows the measurement of the heat production to have been satisfactory.

CORRECTION OF OBSERVED HEAT PRODUCTION

Preparatory to use in the computation of the net energy of a feed it is necessary to apply a correction of the observed heat production to compensate for inequality of time spent by the animal in the standing and the lying positions, a standard day of 12 hours standing and 12 hours lying having been arbitrarily adopted as a feature of the experimental routine.

This correction was made in accord with the recently published method of Fries and Kriss,[4] the details of which will not be repeated here. The method of this correction and the corrected data are as indicated in Table VI.

TABLE VI.—*Observed heat production corrected to 12 hours standing and 12 hours lying*

Period	Observed daily heat production	Hours standing	Difference from 12 hours	Live weight	Hourly correction for position	Correction to standard day	Corrected heat production
	Calories			*Kilograms*	*Calories*	*Calories*	*Calories*
I	11,088.5	10.13	1.87	348.6	24.1	45.1	11,133.6
III	9,723.3	7.05	4.95	353.7	24.1	119.3	9,842.6
V	7,303.7	8.26	3.74	337.1	24.1	90.1	7,393.8
II	10,886.0	9.50	2.50	348.7	24.1	60.3	10,946.3
IV	9,697.6	9.14	2.86	349.4	24.1	68.9	9,766.5
VI	6,795.0	8.98	3.02	329.4	24.1	72.8	6,867.8

METABOLIZABLE ENERGY, HEAT PRODUCTION, AND ENERGY GAIN

In Table VII it is seen that the metabolizable energy varies, in general consistently with the total dry matter, but that as related to the kilograms of dry matter the metabolizable energy of the hay is slightly higher than that of the meal, in harmony with the previously noted difference in digestibility; and that the metabolizable energy on the two higher planes of feed intake, with both feeds, is significantly lower than on the lowest plane of intake. On the two higher planes of intake the metabolizable energy per kilogram of dry matter of both hay and meal agrees very well.

TABLE VII.—*Dry matter, metabolizable energy, heat production, and energy gain*

Feeding stuff and period No.	Dry matter eaten	Metabolizable energy, total	Metabolizable energy per kilogram dry matter	Heat production corrected to standard day	Gain of energy
Alfalfa hay:	*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*
Period I	6.638	13,337	2,009	11,134	+2,203
Period III	5.320	10,650	2,002	9,843	+807
Period V	3.052	6,510	2,133	7,394	−884
Alfalfa meal:					
Period II	6.671	13,238	1,985	10,946	+2,292
Period IV	5.408	10,661	1,971	9,767	+894
Period VI	3.155	6,587	2,088	6,868	−281

[4] FRIES, J. A., and KRISS, M. METABOLISM OF CATTLE DURING STANDING AND LYING. Amer. Jour Physiol. 71: 60–83. 1924.

The figures for heat production, however, when considered in relation to the amount of feed, are less satisfactory, that for Period VI being especially low, with the result that the loss of energy in this period was correspondingly low.

THE HEAT INCREMENT

The heat increment (Table VIII) is the increased heat production due to an increase in the amount of feed, and is expressed, in relation to the kilograms of dry matter of the increase in feed, as the heat increment.

TABLE VIII.—*Heat increment per kilogram of dry matter*

Feeding stuff	Periods compared	Heat increment per kilogram dry matter	Feeding stuff	Periods compared	Heat increment per kilogram dry matter
		Calories			*Calories*
Alfalfa hay	I and III	979	Alfalfa meal	II and IV	934
Do	III and V	1,080	Do	IV and VI	1,287
Do	I and V	1,043	Do	II and VI	1,160
Average		1,034	Average		1,127

With both the hay and the meal there are two high and one low heat-increment values, the high values being those affected by heat production in Periods V and VI, the two involving minimum feed intake.

THE MAINTENANCE REQUIREMENT

The maintenance requirement of energy is computed for each period by subtracting from the heat production the product of the heat-increment value multiplied by the kilograms of dry matter of the feed, as indicated in Table IX.

TABLE IX.—*Maintenance requirement of net energy*

Period No.	Dry matter eaten		Average heat increment per kilogram of dry matter	Total heat increment of ration	Total heat production	Net energy required for maintenance
	Alfalfa hay	Alfalfa meal				
Treatment A:	*Kilograms*	*Kilograms*	*Calories*	*Calories*	*Calories*	*Calories*
I	6.6383		1,034	6,864	11,134	4,270
II		6.6707	1,127	7,518	10,946	3,428
III	5.3203		1,034	5,501	9,843	4,342
IV		5.4078	1,127	6,095	9,767	3,672
V	3.0524		1,034	3,156	7,394	4,238
VI		3.1549	1,127	3,556	6,868	3,312
Average						3,877
Treatment B:						
I	6.6383		979	6,499	11,134	4,635
III	5.3203		979	5,209	9,843	
II		6.6707	934	6,230	10,946	4,716
IV		5.4078	934	5,051	9,767	
Average						4,676

It will be noted that two treatments of the experimental data are given in this table, treatment A representing the usual procedure, and treatment B the same except for the elimination of the data for Periods V and VI, there being, in the writers' opinion, sufficient warrant for pointing out in this way the influence of the data for Periods V and VI, but not sufficient, at this time, to warrant excluding these data from consideration.

The influence of the high heat production in Periods V and VI is reflected, in treatment A, in (1) high average heat-increment values in all periods, but especially in Periods II, IV, and VI, and (2) abnormally low net energy values for maintenance in all periods, but especially in Periods II, IV, and VI. Obviously these latter values are wrong.

Treatment B, exhibiting the computation of the maintenance requirement with the data for Periods V and VI excluded, is consistent within itself and with what is known of the maintenance requirement of steers.

NET-ENERGY VALUES

The net-energy value of the feeds is computed by adding the body gain of energy to the maintenance requirement, or subtracting the body loss of energy from the same. This gives the total amount of net energy for the feed of each period, and this value divided by the kilograms of dry feed involved gives the net energy per kilogram of dry matter (Table X).

TABLE X.—*Net-energy values of alfalfa hay and alfalfa meal*

Feeding stuff and period No.	Dry matter eaten	Metabolizable energy	Average maintenance requirement of net energy	Body gain of energy	Total net energy of ration	Net energy per kilogram of dry matter	Utilization of metabolizable energy
Treatment A:							
Alfalfa hay—	*Kg.*	*Calories*	*Calories*	*Calories*	*Calories*	*Calories*	*Per cent*
I	6.6383	13,337	3,877	+2,203	6,080	916	45.59
III	5.3202	10,650	3,877	+807	4,684	880	43.98
V	3.0524	6,510	3,877	−884	2,993	981	45.98
Average						926	45.18
Alfalfa meal—							
II	6.6707	13,238	3,877	+2,292	6,169	925	46.60
IV	5.4078	10,661	3,877	+894	4,771	882	44.75
VI	3.1549	6,587	3,877	−281	3,596	1,140	54.59
Average						982	48.65
Treatment B:							
Alfalfa hay—							
I	6.6383	13,337	4,676	+2,203	6,879	1,036	51.58
III	5.3202	10,650	4,676	+807	5,483	1,031	51.48
Average						1,034	51.53
Alfalfa meal—							
II	6.6707	13,238	4,676	+2,292	6,968	1,045	52.64
IV	5.4078	10,661	4,676	+894	5,570	1,030	52.25
Average						1,038	52.45

A figure of similar significance, expressing the percentage of utilization of the metabolizable energy, is obtained by dividing the net energy by the metabolizable energy.

Treatment A exhibits considerable diversity in the net energy values for both feeds, but especially for the alfalfa meal—a diversity which does not permit of a distinction between the values of the two feeds; while treatment B gives very closely concordant values and virtually identical figures for the hay and for the meal.

This indication that there is no difference in the nutritive values of the two feeds is in harmony with the conclusion of Fries and Kriss,[1] that the heat production due to the increased muscular activity during the intake of feed and water is normally less in amount than that required to bring the temperature of the ingested feed and water up to that of the body.

SUMMARY

The net-energy value of alfalfa hay of excellent quality is essentially the same when coarsely cut, in a silage cutter, as when finely ground into a meal.

Probably due to difference in the course of digestion, especially with reference to rumination, the finely ground hay was 2.2 per cent less digestible, on a dry-matter basis, than was the coarsely cut hay.

[1] FRIES, J. A., and KRISS, M. Op. cit.

THE VALUE OF LITMUS, BROM-CRESOL PURPLE, AND JANUS-GREEN MILK IN A STUDY OF THE NODULE ORGANISMS OF LEGUMINOSAE[1]

By J. W. STEVENS

Assistant in Agricultural Bacteriology, University of Wisconsin

INTRODUCTION

The value of milk in the study of the nodule bacteria of legumes has been shown by Löhnis and Hansen.[2] They found that in milk, organisms with peritrichous flagella from alfalfa, sweet clover, clover, pea, vetch, lupine, and bean formed a clear serum zone on top 2 to 5 mm. thick, while the milk below remained nearly unchanged; and that organisms of the second group, such as cowpea, peanut, lima bean, and soy bean, which have only one flagellum, formed no serum zone but that later there was a slow digestion of the milk. They also pointed out the value of milk in distinguishing *Bacillus radiobacter* from nodule bacteria of legumes.

The interesting reaction of the nodule bacteria in milk suggested the possibility of improving plain milk by the addition of some indicator, which would show reduction and change in reaction. In an attempt to improve the medium, litmus, brom-cresol purple, and Janus-green milk media were prepared and their value compared with plain milk.

Only fresh skim milk was used as culture medium. This was sterilized in an Arnold steamer at 100° C. for 30 minutes on four successive days. Aqueous solutions of the indicators were sterilized separately and added to the milk. For a comparison of plain and litmus milk four parallel tubes of each were inoculated with the same culture, while with brom-cresol purple and Janus green only two tubes of each culture were used. Pure cultures of nodule bacteria from pea, vetch, clover, garden bean, lima bean, cowpea, soy bean, alfalfa, and sweet clover were used. All cultures were incubated at 28° C.

Of the three indicators, litmus proved by far the most valuable. Brom-cresol purple and Janus green failed to bring out any differences not shown by litmus. Brom-cresol purple showed changes in reaction, while Janus green showed a reduction. As Janus green remains reduced permanently it is of less diagnostic value than litmus. Brom-cresol purple was not reduced by any of the nodule organisms. This indicator retarded the growth of the organisms, in some cases completely. High concentrations of litmus in milk had no noticeable inhibitive effect on the growth of these organisms, and they showed changes in reaction equally as well as brom-cresol purple, and in indicating reduction they were as satisfactory as Janus green.

After one to three weeks most of the cultures showed an alkaline reaction in brom-cresol-purple milk. However, after five weeks some

[1] Received for publication May 6, 1925; issued January, 1926. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] LÖHNIS, F., and HANSEN, R. NODULE BACTERIA OF LEGUMINOUS PLANTS. Jour. Agr. Research 20: 543-556, illus. 1921.

of the alfalfa and sweet clover strains produced an acid reaction. Some of the cultures showed no change during eight weeks. Perhaps this was due to a failure of the organisms to grow. These data are shown in Table I.

TABLE I.—*Characteristics of nodule bacteria in milk, with various indicators, after five weeks at 28° C.*

Strain and No.	Plain milk	Litmus milk		Brom-cresol purple milk reaction
		Reaction	Reduction	
Sweet clover, 110, 111, 112	Serum zone	Acid	At bottom	Acid.
Alfalfa, 106, 107, 108	do	do	do	Do.
Alfalfa, 100, 103, 104	do	do	do	No change.
Alfalfa, 101, 102, 105	do	do	do	Slightly alkaline.
Clover, 121, 122	do		Throughout	Very alkaline.
Clover, 123, 124, 125	do	Alkaline	None	Do.
Garden pea, 130	do	Very alkaline	Slight	
Garden pea, 132	do	do	None	Do.
Garden pea, 131	do		Throughout	Slightly alkaline.
Vetch, 134	do	Very alkaline	None	Very alkaline.
Garden bean, 140	do	do	do	Alkaline.
Cowpea, 143	None	do	do	No change.
Lima bean, 142	do	do	do	Alkaline.
Soy bean, 161, 164, 166	do	do	do	No change.
Soy bean, 160, 162, 163, 165	do	do	do	Very alkaline.

Most of the organisms reduced Janus green to a permanent pink color in from one to three weeks, as shown in Table II. Some cultures reduced this indicator only at the bottom, while others reduced it throughout. Soy-bean, cowpea, and lima-bean nodule organisms were least active in reducing the indicator. Three strains of soy-bean nodule organisms produced no change within 39 days. Very little, if any, change in reaction was ever noted in the Janus-green culture.

TABLE II.—*Effect of the growth of the nodule bacteria on Janus-green milk (duplicate tubes)*

Strain and No.	Changes produced after—	
	7 days	39 days
Sweet clover, 110, 111, 112	Pink at bottom	Pink.
Alfalfa, 100	Pink	Do.
Alfalfa, 106, 107, 108	Pink at bottom	Do.
Alfalfa, 103	do	Pink at bottom.
Alfalfa, 101, 102, 104, 105	No change	Do.
Clover, 121	Light pink	Pink.
Clover, 123	Whitish green	Do.
Clover, 124	Pink	Do.
Clover, 125	Light pink	Do.
Garden pea, 131	Pink	Do.
Garden pea, 132	No change	Do.
Vetch, 134	Pink	Do.
Garden bean, 140	do	Do.
Lima bean, 142	No change	Pink at bottom.
Soy bean, 161, 164, 166	do	No change.
Soy bean, 160, 162, 163	do	Pink at bottom.
Soy bean, 165	do	Pink.

The results obtained with litmus milk were more specific and were superior to those obtained with plain milk. As will be seen in Table I, litmus milk not only showed all the characteristics brought out by plain milk, but in addition showed changes in reaction and reduction.

According to change in reaction, the cultures fall into two groups: Acid production with strong reduction, and alkaline production with little or no reduction.

The cultures from alfalfa and sweet-clover nodules were the only ones that showed an acid reaction. Within the first few days after inoculation the organisms produced an alkaline reaction in milk, but later they began to reduce the litmus, and still later the milk began to turn pink. The rate of acid production was much faster with freshly isolated strains. The tubes of milk usually remained reduced at the bottom, while the upper layer was pink. Some cultures kept the litmus reduced completely below the serum zone.

The cultures of the second group—which included pea, vetch, clover, bean, lupine, cowpea, lima-bean and soy-bean organisms—produced alkaline reactions (Table I). The milk cultures showed a change in reaction within a few days after inoculation. The most marked changes were produced by soy-bean, lima-bean, cowpea, and pea cultures. Some clover and pea strains reduced the litmus completely after about two weeks. These were cultures which had been cultivated in the laboratory for a number of years. Other clover, pea, and bean strains reduced the litmus slightly, usually at the bottom of the tube. Cultures of cowpea, lima bean, and soy bean did not reduce the litmus. A film growth over the surface was formed by some cultures of clover, pea, and vetch. This film formation was especially noticeable with strains which showed a marked reduction of the litmus.

The characteristic reaction of *Bacillus radiobacter* is shown quite as well in litmus milk as in milk without the indicator.

The effect of the growth of these cultures on the hydrogen-ion concentration of the milk after 47 days was studied. The determinations reported below were made by the electrometric method:

	P_H
Uninoculated	6.55
Alfalfa, 108	5.18
Do. 109	5.31
Soy bean, 164	7.75
Do. 168	8.37
Lupine, 150	7.55
Lima bean, 142	7.85
Cowpea, 143	8.08

The alkaline reaction produced by the growth of soy-bean, cowpea, and lima-bean nodule organisms in milk probably accounts for the liquefying effect on the milk casein noted by Löhnis and Hansen.[1] These investigators noted a slow digestion of the casein by these organisms after a long time. Preliminary experiments by the writer indicate that the nodule organisms have very little, if any, effect on the milk casein. However, the alkaline reactions produced by soy-bean, cowpea, and lima-bean nodule organisms, as shown above, are sufficient to cause a slow dissolving of the milk casein, with a resulting decrease in opacity of the milk.

A serum zone was formed by the alfalfa, sweet-clover, clover, pea, vetch, and bean cultures. Clover, pea, and vetch cultures would often produce a serum zone in two or three days, while some strains of alfalfa, sweet-clover and bean organisms would often require

[1] Op. cit.

from two to four weeks. The cowpea, lima-bean, and soy-bean cultures did not form a serum zone. The rate of serum-zone formation is influenced greatly by the amount of inoculum.

The behavior of the various cultures of nodule-forming bacteria in litmus milk was of considerable value in separating strains. Differences were noted in rate of serum-zone formation, depth of zone, amount of reduction, and in change of reaction among organisms of a single cross-inoculating group. Probably the ability of these organisms to change the reaction of the medium in which they grow is an adaptation to their natural environment and has some relation to their life functions.

CONCLUSIONS

The litmus milk brings out characteristics of the nodule bacteria not shown by plain milk. On the basis of reaction and reduction, it separates these organisms into two groups. This separation is more marked with freshly isolated cultures than with cultures carried in the laboratory for a long time.